PHILOSOPHICAL TRANSACTIONS.

I. *On the present Position of the Question of the Sources of the Nitrogen of Vegetation, with some new Results, and preliminary Notice of new Lines of Investigation.*

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Received, Part 1, July 20, 1887; Parts 2 and 3, May 3, 1888—Read May 17, 1888.

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Introduction.

A GREAT part of this paper was written in the spring of 1886, but its completion was unavoidably delayed. This has, however, not been altogether without advantage. Thus, in the first place, at the *Naturforscher-Versammlung*, held in Berlin, in September, 1886, the greater part of the sittings of two days was devoted, in the Section of *Landwirthschaftliches Versuchs-Wesen*, to the discussion of the subject from various points of view, one of ourselves taking part; and as it seemed desirable that the results and conclusions then brought forward by others should be considered, we have waited for the publication of the exact figures in some cases. Again, since the Berlin meeting, M. BERTHELOT has published some further results, to which reference should be made. And lastly, we are now enabled to give further new results of our own.

In Part 2 of the 'Philosophical Transactions' for 1861, a paper was given, by ourselves and the late Dr. PUGH, "*On the Sources of the Nitrogen of Vegetation, with special reference to the question whether plants assimilate free or uncombined Nitrogen.*" Since that time, the question of the sources of the nitrogen of vegetation has continued to be the subject of much discussion, and also of much experimental enquiry, both at Rothamsted and elsewhere. Until quite recently, the controversy has chiefly been as to whether plants directly assimilate the free nitrogen of the atmosphere; but, during the last few years, the discussion has assumed a somewhat different aspect. The question still is whether the free nitrogen of the air is an important source of the nitrogen of vegetation; but whilst few now adhere to the view that the higher chlorophyllous plants directly assimilate free nitrogen, it is, nevertheless, assumed to be brought under contribution in various ways—coming into combination within the soil, under the influence of electricity, or of micro-organisms, or of other low forms, and so indirectly serving as an important source of the nitrogen

of plants of a higher order. Several of the more important of the investigations in the lines here indicated seem to have been instigated by the assumption that compensation must be found for the losses of combined nitrogen which the soil sustains by the removal of crops, and also for the losses which result from the liberation of nitrogen from its combinations under various circumstances.

At the meeting of the American Association for the Advancement of Science, held at Montreal in 1882, we gave a paper entitled—"*Determinations of Nitrogen in the soils of some of the Experimental Fields at Rothamsted, and the bearing of the results on the question of the Sources of the Nitrogen of our crops;*" and again, at the Meeting of the British Association, held at Montreal, in 1884, we gave further results on the subject, in a paper—"*On some points in the Composition of Soils; with results illustrating the Sources of the Fertility of Manitoba Prairie Soils.*" *

It is the object of the present paper to summarise some of our own more recently published results bearing on various aspects of the subject, to put on record additional results, to give a preliminary notice of new lines of enquiry, and to discuss the evidence so adduced with reference to the results and conclusions of others which have recently been put forward, as above alluded to.

PART I.

Results relating to other Sources than Free Nitrogen.

1. *Summary of previously published Rothamsted Results chiefly relating to nitric acid in soils and subsoils.*

Before directing attention to the new results it will be desirable, with the view of bringing out their significance the more clearly, to give a brief *résumé* of our previous results and conclusions bearing on the subject.

In the last mentioned paper, after reviewing previously existing evidence as to the sources of the nitrogen of crops, we concluded, as we had done before, that, excepting the small amount of combined nitrogen annually coming down in rain, and the minor aqueous deposits from the atmosphere, the source of the nitrogen of vegetation was, substantially, the stores within the soil and subsoil, whether derived from previous accumulations or from recent supplies by manure.

Results of determinations of the nitrogen as nitric acid, in soils of known history as to manuring and cropping, and to a considerable depth, in some cases to 108 inches, were given, which showed that the amount of nitrogen in the soil in that form was much less after the growth of a crop than under corresponding conditions without a crop. In the case of gramineous crops, the evidence pointed to the conclusion that most, if not the whole, of their nitrogen was taken up as nitric acid from the soil and

* Afterwards revised and published in the 'Transactions of the Chemical Society' for June, 1885.

subsoil. In the case of leguminous crops again, the evidence was in favour of the supposition that, in some cases, the whole of the nitrogen had been taken up as nitric acid, but that in others that source was inadequate.

The results further showed that, under otherwise parallel conditions, there was very much more nitrogen as nitric acid, in soils and subsoils, down to a depth of 108 inches, where leguminous than where gramineous crops had for some time been grown.

Table II., p. 6, gives, in a condensed form, the most important of the previously published results relating to this branch of the subject. It shows the amounts of nitrogen as nitric acid, calculated *per acre*, in lbs., according to determinations made in samples of soil collected in 1883, at each of 12 depths of 9 inches each, that is down to 108 inches in all, under the following conditions :—

1. Where wheat had been grown in alternation with fallow, without any manure, since 1850 ; that is for more than 30 years.

2. On a plot where mineral manures had occasionally been applied, but no nitrogen for more than 30 years ; where *Trifolium pratense* had been sown 12 times during the 30 years, 1848–77, but in 8 out of the last 10 trials the plant had died off in the winter or spring succeeding the sowing, in 4 without giving any crop, and in the other 4 yielding very small cuttings ; and where, consequent on the failure of the clover, during the 30 years 1 crop of wheat and 5 of barley had been taken, and the land had been 12 years left fallow. *Trifolium repens* was then sown, namely, in 1878, 1880, 1881, and 1883 ; and it yielded crops in 1879, 1881, and 1882, but none in 1883, when the soil samples were taken.

3. On 2 plots with the same previous history as that of the *Trifolium repens* plots prior to 1878, but where *Vicia sativa* has since been sown ; and where, notwithstanding the previous failure of the red clover, the *Vicia* yielded fair crops in 1878, 1879, 1880, and 1881, and large crops in 1882, and in 1883, before the soil samples were taken.

We are now able to give amended, and more complete estimates, of the amounts of nitrogen removed per acre in the produce from the different plots, both during the preliminary period up to 1877 inclusive, and during the period of the direct experiments with the various leguminous plants. As each leguminous plot is only $\frac{1}{84}$ of an acre in area, it is obvious that calculations of the produce, per acre, can only be approximately correct. We have, therefore, in each case taken the average yield of three plots, 4, 5, and 6, with one and the same plant, but with somewhat different mineral manures. Thus, plot 4 has received a mixture of superphosphate of lime and potash sulphate ; plot 5 a mixture of potash, soda, and magnesia salts ; and plot 6 the same as plot 5, with superphospate of lime in addition. Direct determinations of nitrogen have, in almost all cases, been made ; and we believe that the estimates of it per acre may be considered as close approximations to the truth, and at any rate quite sufficiently so for the purposes of illustration and of argument for which they are used. The results are given in the following Table (I.).

TABLE I.—Estimated yield of Nitrogen per acre, in lbs., in wheat alternated with fallow, and in various leguminous crops without nitrogenous manure.

	Unmanured.	Mineral manures only.	
	Fallow-wheat.	*Trifolium repens.*	*Vicia sativa.*
Preliminary period:—Wheat and fallow, 27 years, 1851–77. Red clover, &c., &c., 29 years, 1849–77.			
Average per acre per annum	lbs. 17	lbs. 32	
Experimental period.			
	lbs.	lbs.	lbs.
1878	29	0	51
1879	Fallow	82	46
1880	24	0	58
1881	Fallow	8	65
1882	18	74	146
1883	Fallow	0	101
Total, 6 years	71	164	467
Average annual	12	27	78

The next Table (II.) shows the amounts of nitrogen as nitric acid found in the soils and subsoils of the several plots under the conditions above described.

TABLE II.—Nitrogen as Nitric Acid per acre, lbs., in the soils and subsoils of some experimental plots, without nitrogenous manure for more than 30 years. Hoosfield, Rothamsted. Soil samples collected July 17–26, 1883.

Depths.	Unmanured.	Series I. Mineral manures only.			*Trifolium repens.* + or –. Wheat land.	+ or –. *Trifolium repens.*	
	Wheat-fallow land.	*Trifolium repens.* Plot 4.	*Vicia sativa.* Plot 4.	*Vicia sativa.* Plot 6.		*Vicia sativa.* Plot 4.	*Vicia sativa.* Plot 6.
inches.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1– 9	19·85	30·90	12·16	10·22	+ 11·05	– 18·74	– 20·68
10– 18	8·05	27·73	4·11	2·72	+ 19·68	– 23·62	– 25·01
19– 27	2·47	8·44	1·37	1·08	+ 5·97	– 7·07	– 7·36
28– 36	2·70	7·64	1·67	1·52	+ 4·94	– 5·97	– 6·12
37– 45	1·62	9·07	4·58	2·51	+ 7·45	– 4·49	– 6·56
46– 54	3·57	8·77	6·37	4·42	+ 5·20	– 2·40	– 4·35
55– 63	3·84	7·92	7·16	4·52	+ 4·08	– 0·76	– 3·40
64– 72	2·28	8·34	5·95	4·92	+ 6·06	– 2·39	– 3·42
73– 81	1·48	8·27	4·54	4·81	+ 6·79	– 3·73	– 3·46
82– 90	1·76	9·95	5·32	5·14	+ 8·19	– 4·63	– 4·81
91– 99	2·94	9·16	5·66	6·40	+ 6·22	– 3·50	– 2·76
100–108	1·84	9·51	5·32	6·46	+ 7·67	– 4·19	– 3·05
Total	52·40	145·70	64·21	54·72	+ 93·30	– 81·49	– 90·98

These wheat-fallow and leguminous plots are absolutely adjoining; and by their previous treatment their surface soils had become extremely poor in nitrogen. The results have been discussed in detail in the paper in the Transactions of the Chemical Society, and must only be briefly summarised here. Table I. shows that, for about 30 years, the *Trifolium repens* soil had yielded in crops nearly twice as much nitrogen per acre as the wheat-fallow soil. Yet it is seen that, whilst the wheat-fallow soil contained, down to the depth of 9 feet, only 52·4 lbs. of nitrogen as nitric acid per acre, the *Trifolium repens* soil contained 145·7 lbs. to the same depth. In other words—the *Trifolium repens* soil, from which so much more nitrogen had been removed, contained 93·3 lbs. more nitrogen as nitric acid than the wheat-fallow soil.

Now, excepting that the leguminous crop soil had received mineral manures, and the wheat soil had not, the characteristic difference in the history of the two plots was, that the one had grown a gramineous crop alternately with fallow for more than 30 years, and the other had, during the same period, besides growing 6 gramineous crops, and being frequently fallow, been sown 12 times with red clover, and, during the immediately preceding 6 years, 4 times with white clover. That is to say, the chief distinction was, that the one plot had, especially in the earlier and the later years, grown a leguminous crop, whilst the other had not; and it is under these circumstances that the leguminous crop soil is found to contain, down to 108 inches, nearly 3 times as much nitrogen as nitric acid as the gramineous crop soil.

The difference is the greatest near the surface, but it is very considerable down to the lowest depth. Hence it is obvious that any loss by drainage would be much the greater from the *Trifolium* plot, and so the difference between the two plots was probably in reality greater than the figures show. In both cases the actual amount is the greatest near the surface, indicating more active nitrification; and the excess is much the greater in the *Trifolium repens* soil, doubtless due to more nitrogenous crop-residue from the leguminous than from the gramineous crop. Indeed, about 74 lbs. of nitrogen had been removed in the *Trifolium repens* crop in 1882, and none in 1883, the year of the soil collections. On the other hand, only about one-fourth as much was removed in the wheat crop of 1882, and the land was fallow in 1883. Unless, however, there was considerably more nitrogen in the crop-residue than in the removed crops of the *Trifolium repens*, the excess of 93 lbs. of nitrogen as nitric acid found in the *Trifolium repens* soil, together with the increased amount lost by drainage, could not have had its source entirely in the nitrification of recent nitrogenous crop-residue. Some of the increased amount in the lower layers was indeed doubtless due to washing down from the surface. But as, notwithstanding much more nitrogen had been removed in crops from the leguminous than from the gramineous crop soil during the previous 30 years, the surface soil of the leguminous plots remained slightly richer in nitrogen than that of the gramineous plot, it cannot be supposed that the whole of the nitrogen of the crop, and of the nitric acid found, had its origin in the surface soil. If, therefore, nitrogen has not been derived from the atmosphere, the conclusion must be that some has come from the subsoil.

The indication was that nitrification had been more active under the influence of the leguminous than of the gramineous growth and crop-residue. In fact, under the influence of leguminous growth, not only will there be increased nitrogenous matter for nitrification, but it would seem that the development of the nitrifying organisms will be favoured. The question is, therefore, whether part of the result be not due to the passage downwards of the nitrifying organisms, and the nitrification of the nitrogen of the subsoil.

The alternative was suggested, that the soil and subsoil might still be the source of the nitrogen of the crops, but that the plants may take up, at any rate part of their supply, in other forms than as nitric acid—as ammonia, or as organic nitrogen, for example. It was pointed out that fungi do take up both organic carbon and organic nitrogen; but that, whilst existing direct experimental evidence was conflicting as to whether green leaved plants even assimilate carbon taken up by their roots as carbonic acid, the evidence was even less conclusive as to whether they take up either organic carbon or organic nitrogen as such from the soil. To this question we shall recur further on.

The next point is to compare the amount of nitrogen as nitric acid found in the *Vicia sativa* soils with that in the *Trifolium repens* soil. In the first place it is to be observed, that whilst from the *Trifolium repens* plot only 164 lbs. of nitrogen had

been removed in the crops during the five years to 1882 inclusive, from the *Vicia sativa* plots 366 lbs. had been removed during the same period. Further, whilst from the *Trifolium repens* plot there was no nitrogen removed in 1883, the year of soil sampling, from the *Vicia* plots 101 lbs. were removed in the crop just before the soil sampling. It is seen that, under these circumstances, there remained, per acre, in one of the *Vicia* plots 81·5 lbs., and in the other 91 lbs., less nitrogen as nitric acid to the depth examined than in the *Trifolium repens* soil.

If we confine attention only to the amount of nitrogen removed in the *Vicia* crops in the year of the soil sampling, and assume that there had been only as much at the disposal of the plant as in the case of the *Trifolium* plot, it is obvious that the deficiency in the *Vicia* soils very nearly corresponds with the amount removed in the crop, which was about 100 lbs. Indeed, it may safely be concluded that most, if not the whole, of the nitrogen of the *Vicia* crops had been taken up as nitric acid.

But there had probably been more loss by drainage from the *Trifolium* plot without growth than from the *Vicia* plots with growth, and with, at the same time, much more upward passage and evaporation.

It must also be borne in mind, that the *Vicia* plots had, during the preceding 5 years, 1878 to 1882, yielded an average of more than 70 lbs. of nitrogen per acre, and in the immediately preceding year (1882), 146 lbs. Further, the amounts taken up by the plants each year must have been much greater than the amounts removed in the crops; for there must have been annually a large crop-residue, which would yield nitric acid for succeeding crops. Much of these large amounts of nitrogen must obviously have had some other source than the original surface soil, since it gained rather than lost under the treatment. If this source were not the atmosphere, but the subsoil, it must have been taken up, either as nitric acid, as some other product of the change of the organic nitrogen of the subsoil, or as organic nitrogen itself. Further, as the *Vicia* crops were large in the previous year, 1882, so also would their nitrogenous crop-residue be large, and contribute correspondingly large amounts of nitric acid for the crops of 1883. But the crops of 1883 were also large, and they, in their turn, would leave correspondingly large nitrogenous crop-residues; leaving a large proportion of the amount of nitrogen removed in the crops to be otherwise provided for than by previous residue.

Lastly in reference to these experiments, it is seen that at each of the 12 depths, down to 108 inches, the *Vicia* plots where there had been growth, contained less nitric-nitrogen than the *Trifolium repens* plot where there had been no growth. The difference is much the greatest in the first 18 inches, within which the *Vicia* throws out by far the larger amount of root; but it is very distinct below this point, and the supposition is that, under the influence of the growth of the *Vicia*, water had been brought up from below, and with it nitric acid. In fact, compared with the *Trifolium repens* plot, the mean for the two *Vicia* plots showed less water in the soil down to

108 inches, corresponding to between 6 and 7 inches of rain, or to between 600 and 700 tons of water per acre.

After this summary of previously published results we may now turn to the consideration of new results of the same kind.

2. *New Determinations of Nitric Acid in Soils and Subsoils.*

The plots experimented upon are in the same series, with the same previous history, as those already referred to. *Trifolium repens* was again selected as the weak and superficially rooting plant; *Melilotus leucantha* was taken as a deeper and stronger rooting one; and *Medicago sativa,* or lucerne, as a still deeper and still stronger rooting plant. Samples of soil were taken at the end of July and the beginning of August, 1885, from 2 places on each plot, and in each case, as before, to 12 depths of 9 inches each, equal to a total depth of 108 inches or 9 feet.

The following table (III.) shows the estimated yields of nitrogen per acre in the different crops during the experimental period from 1878 to 1885, the year of soil sampling, inclusive. The yields during the preliminary period have been already given in Table I.

TABLE III.—Estimated yield of Nitrogen per acre, in lbs., in wheat alternated with fallow, and in various leguminous crops, without nitrogenous manure.

	Unmanured.	Mineral manures only.		
	Fallow-wheat.	*Trifolium repens.*	*Melilotus leucantha.*	*Medicago sativa.*
	lbs.	lbs.	lbs.	lbs.
1878	29	0	53	Not sown
1879	Fallow	82	130	0
1880	24	0	36	28
1881	Fallow	8	60	28
1882	18	74	145	111
1883	Fallow	0	27	143
1884	29	0	56	337
1885	Fallow	97	58	233*
Total 8 years . .	100	261	565	880
Average annual .	12	33	71	(110)

Thus, the wheat plot was again fallow when sampled; the total yield of nitrogen in the crops in the 8 years was only 100 lbs. per acre, and the average annual yield little more than 12 lbs.

The *Trifolium repens* plots, after giving no crop in either 1883 or 1884, yielded produce containing nearly 100 lbs. of nitrogen in 1885, before the soil sampling; the total yield of nitrogen in the 8 years was 261 lbs., and the average annual yield 33 lbs.

* First and second crops only; a third crop, cut after the soil sampling, yielded 37 lbs. nitrogen.

The deeper rooting, and freer growing *Melilotus leucantha* gave more or less produce in each year of the eight, large crops in 1879 and 1882, a total yield of nitrogen over the eight years of 565 lbs. per acre, and an average annual yield of 71 lbs.

Lastly, the still deeper rooting, and still freer growing *Medicago sativa*, sown first in 1879, gave no crop in that year, only small crops in 1880 and 1881, and then rapidly increasing amounts, until the yield of nitrogen was estimated at 337 lbs. per acre in 1884, and at 233 lbs. in 1885 before the soil sampling, and 37 lbs. afterwards, making a total for that year of 270 lbs. The total yield of nitrogen in the 6 years, prior to the soil sampling, was 880 lbs.; giving an average over the 8 years of 110 lbs., or over the 6 years when there was any crop, of 147 lbs. of nitrogen per acre per annum.

Table IV. shows the amounts of nitrogen as nitric acid found in the soils and subsoils of the different plots—in all cases calculated into lbs. per acre.

TABLE IV.—Nitrogen as Nitric Acid per acre, lbs., in the soils and subsoils of some experimental plots, without nitrogenous manure, for more than 30 years. Hoosfield, Rothamsted. Samples collected July 29 to August 14, 1885.

Depths.	Unmanured.	Series I. Mineral manures only.			*Trifolium repens.* + or − Wheat land.	+ or − *Trifolium repens.*	
	Wheat-fallow land.	*Trifolium repens.* Plot 5.	*Melilotus leucantha.* Plot 5.	*Medicago sativa.* Plot 5.		*Melilotus leucantha.* Plot 5.	*Medicago sativa.* Plot 5.
inches.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1– 9	17·44	11·50	4·35	8·88	− 5·94	− 7·15	− 2·62
10– 18	3·67	1·38	1·40	1·11	− 2·29	+ 0·02	− 0·27
19– 27	2·76	0·90	2·12	0·78	− 1·86	+ 1·22	− 0·12
28– 36	2·16	1·86	2·94	0·81	− 0·30	+ 1·08	− 1·05
37– 45	1·68	7·08	5·22	0·99	+ 5·40	− 1·86	− 6·09
46– 54	1·47	11·31	6·21	0·93	+ 9·84	− 5·10	−10·38
55– 63	1·77	13·14	7·95	0·57	+11·37	− 5·19	−12·57
64– 72	1·83	12·63	10·08	0·81	+10·80	− 2·55	−11·82
73– 81	2·29	11·19	9·66	0·70	+ 8·90	− 1·53	−10·49
82– 90	2·01	10·70	9·16	0·61	+ 8·69	− 1·54	−10·09
91– 99	1·98	11·08	8·83	0·44	+ 9·10	− 2·25	−10·64
100–108	2·06	9·96	10·12	0·41	+ 7·90	+ 0·16	− 9·55
Total	41·12	102·73	78·04	17·04	+61·61	−24·69	−85·69
Summary and control.							
1– 9	17·44	11·50	4·35	8·88	− 5·94	− 7·15	− 2·62
10– 18	3·67	1·38	1·40	1·11	− 2·29	+ 0·02	− 0·27
Mixture of 19–108 inches	20·63	88·02	73·21	6·97	+67·39	−14·81	−81·05
Total	41·74	100·90	78·96	16·96	+59·16	−21·94	−83·94

The determinations of nitric acid in the soil extracts, the results of which are recorded in the table, as well as those given in Table II., were made in the Rothamsted Laboratory by Mr. D. A. LOUIS, by SCHLŒSING'S method, as nitric oxide by the reaction with ferrous salts. For each of the twelve depths a mixture of the samples from the two holes was prepared, and in each of these mixtures duplicate determinations of nitric acid were made. As a control, determinations were also made in a mixture of the samples from the 10 lower depths, the third to the twelfth inclusive, and the results are given at the foot of the table.

The first point to remark is, that there was much less nitrogen as nitric acid in the *Trifolium repens* soil in 1885, after the removal of nearly 100 lbs. of nitrogen in the crops, than in 1883, when no crop had grown. The deficiency is the greatest in the 2 upper layers, but it extends to the fifth depth, amounting to that point, which represents the range of the direct or indirect action of the superficial roots of the plant, to about 61 lbs. Below the range of this action, however, there is even more nitrogen as nitric acid in 1885 than 1883; due doubtless in part to percolation from above during the two preceding seasons without growth, and possibly in part to percolation of the nitrifying organisms, and nitrification of the nitrogen of the subsoil.

Let us now turn to the results obtained on the *Melilotus leucantha* plot. As shown in Table III., it is estimated that, in 1882 as much as 145 lbs. of nitrogen was removed in the crop, and samples of soil taken that autumn, to the depth of 6 times 9 inches, or 54 inches in all, showed only 8·45 lbs. of nitrogen as nitric acid remaining, which was 17·8 lbs. less than was found to the same depth in the *Trifolium repens* plot which had yielded only 74 lbs., or only about half as much in the crop as the *Melilotus*.

After 1882, however, the produce of the *Melilotus* declined very much, and in 1885 the yield of nitrogen in the crop was estimated at only 58 lbs., against 97 lbs. estimated to have been removed in the *Trifolium repens* crop. Under these circumstances the *Trifolium repens* soil shows even rather less nitric acid than the *Melilotus* soil, in the second, third, and fourth depths, which comprise the chief range of action of the *Trifolium repens* roots. At every depth below the fourth, however (except the 12th, where the difference is very small), there is notably less nitrogen as nitric acid in the *Melilotus* than in the *Trifolium repens* soil, the *Melilotus* having yielded so much more in its crops in the preceding years than the *Trifolium repens*. To the total depth of 108 inches there was 24·69 lbs. less nitric nitrogen remaining in the *Melilotus* than in the *Trifolium repens* soil.

Admittedly we cannot know what was the stock of nitric nitrogen in either soil at the commencement of the growth of the season. But as during the 8 years 565 lbs. of nitrogen were removed in the *Melilotus* crops, against only 261 lbs. in the *Trifolium repens*, or more than twice as much in the *Melilotus* as in the *Trifolium repens*, it may be supposed that the *Melilotus* would both leave more nitrogenous crop-residue

for nitrification, and with its deeper roots, would each year the more exhaust the nitric nitrogen especially of the lower layers. Hence, notwithstanding the much lower yield of nitrogen in the *Melilotus* than in the *Trifolium repens* crop in 1885, the lower layers of the *Melilotus* soil contained less nitric acid than those of the *Trifolium repens* soil. There can, indeed, be no doubt, that the *Melilotus* derived at any rate much of its nitrogen from nitric acid, either within the actual range of its roots, or within the range of their action in causing the passage upwards of water with its dissolved contents. Still, the figures show, that with the comparatively limited growth in the recent years, there remained per acre about 56 lbs. of nitrogen as nitric acid in the 6 lower depths of the *Melilotus* soil.

But by far the most striking results in the Table are those relating to the *Medicago sativa* (lucerne) soil, and to the comparison between the amounts of nitric nitrogen in the soil of the shallow rooting and weakly growing *Trifolium repens* and those in the soil of the very deep and strong rooted, and very free growing lucerne.

Table III. shows that the estimated yields of nitrogen per acre in the lucerne were in the 6 years, 1880–1885, respectively as follows :—28 lbs., 28 lbs., 111 lbs., 143 lbs., 337 lbs., and 233 lbs. That is to say, with the increasing root range, and consequently increased command of the stores of the soil and subsoil, the yield of nitrogen in the crop increased from 28 lbs. in the first and second years, to 337 in the fifth year ; declining, however, somewhat in the sixth year, 1885, and it did so still further in 1886.

It is seen that under these circumstances of very large yields of nitrogen in the crops, there is, at every one of the twelve depths, less, and at most very much less nitrogen as nitric acid remaining in the soil than where so much less nitrogen had been removed in the *Trifolium repens* crops. The difference is distinct even in the upper layers, but it is very striking in the lower depths. Thus, there is, on the average, not one-twelfth as much nitric nitrogen in the lower ten depths of the deep rooting and high nitrogen-yielding *Medicago sativa* soil, as in those of the shallow rooting and comparatively low nitrogen-yielding *Trifolium repens* soil. Indeed, the nitric acid is nearly exhausted in the deep rooting *Medicago sativa* plot ; there remaining, to the total depth of 9 feet, only about 17 lbs. of nitric nitrogen against more than 100 lbs. to the same depth in the *Trifolium repens* soil. The total deficiency of nitric nitrogen in the *Medicago* as compared with the *Trifolium repens* soil, is seen to be 85·69 lbs. according to one set of determinations, and 83·94 lbs. according to the other.

As already said, we cannot know what was the stock of nitric nitrogen in the soil at the commencement of the growth of the season, or the amount formed during the growing period. But with so much more *Medicago* growth for several previous years, it seems reasonable to assume that there would be much more nitrogenous crop-residue for nitrification than in the case of the *Trifolium repens* plot.

But even supposing, for the sake of illustration, that each year's growth would leave crop-residue yielding an amount of nitrogen as nitric acid for the next crop, or succeeding crops, approximately equal to the amount which had been removed in the

crop, the increasing amounts of nitrogen yielded in the crops from year to year could not be so accounted for; and there would remain the amount of nitrogen in the crop-residue itself, still to be provided in addition. In fact, assuming the proportion of nitrogen in the crop-residue to that in the removed crop to be as supposed in the above illustration, nearly 700 lbs. of nitrogen would have been required for the *Medicago* crop and crop-residue of 1884; or if we assume the nitrogen in the residue to be only half that in the crop, about 500 lbs. would have been required. Doubtless, however, some of the nitrogenous crop-residue would accumulate from year to year.

The results can leave no doubt that the *Trifolium repens*, the *Melilotus leucantha*, and the *Medicago sativa*, have each taken up much nitrogen from nitric acid within the soil. But, at any rate so far as the *Medicago* is concerned, there is nothing in the figures to justify the conclusion that the whole of its nitrogen can have been so derived. It is obvious that if nitric acid were the source of the whole there must have been a great deal formed by the nitrification of the nitrogen of the subsoil. The alternative is—provided the atmosphere be not the source—that the deep-rooted plant takes up nitrogen from the subsoil in some other way.

3. *Percentage of Nitrogen in the Surface Soils of the Experimental Plots.*

It has been stated in general terms that, although much more nitrogen had been removed from the leguminous crop soils than from the fallow-wheat land, for nearly 30 years prior to the commencement of the experiments with various leguminous plants in 1878, yet the leguminous crop surface soil remained rather richer in nitrogen than the fallow-wheat soil. It has also been stated that, during the subsequent years of experiment with the various leguminous plants, the surface soil had gained rather than lost nitrogen. It will be well to give a summary of the actual experimental results relating to these points.

In the first place it is to be borne in mind, as Table I. (p. 5) shows, that whilst over the 27 years 1851–1877 inclusive, only about 17 lbs. of nitrogen were removed per acre per annum in the wheat grown in alternation with fallow, there was, over the 29 years, 1849–1877 inclusive, an average of about 32 lbs., or nearly twice as much, removed from the adjoining clover plots.

During the years 1878, 1879, and 1880, the yield of nitrogen in the wheat was about the same as the average of the preceding 27 years; whilst in most of the leguminous crops the yield was more than over the preceding 29 years. In the autumn of 1880 all the plots were ploughed up, and at the end of March, 1881, before resowing, soil samples were taken from five places on the leguminous crop-land, and also from five on the portion of the wheat land which was then fallow. The samples were, in each case, taken to the depth of 3 times 9 inches, or 27 inches in all.

It has already been stated that no nitrogenous manure had been applied to either the fallow-wheat, or the leguminous crop land, for more than 30 years; but that to

the leguminous crop land different mineral manures had occasionally been applied.

They were as follows :—

Plot 2. Superphosphate of lime.

Plot 3. Sulphate of potash.

Plot 4. Sulphate of potash and superphosphate.

Plot 5. Salts of potash, soda, lime, and magnesia.

Plot 6. As 5, with superphosphate in addition.

Soil samples were taken from each of the 5 plots, and also from 5 places directly opposite to them on the immediately adjoining fallow-wheat land. The following table (V.) shows the percentage of nitrogen in the fine sifted surface soil, 9 inches in depth, as dried at 100° C., in the sample from each plot. It also shows the mean for the five plots, the mean of determinations made on mixtures from the five plots, and the general means of these two sets of determinations. It should be added that each figure given is the mean of three or more determinations made by the soda-lime method.

TABLE V.—Nitrogen per cent. in soil samples collected March, 1881.

	Nitrogen per cent. in fine surface soil, 9 inches deep. Calculated on the soil as dried at 100° C.	
	Leguminous crop soils.	Fallow-wheat soil.
	Per cent.	Per cent.
Plot 2	0·1064	0·0938
" 3	0·1036	0·0930
" 4	0·0950	0·0931
" 5	0·1100	0·0957
" 6	0·1156	0·1007
Means	0·1061	0·0953
On mixture	0·1055	0·0984
General means	0·1058	0·0969

There can be no doubt that both the leguminous crop and the fallow-wheat surface soils had lost nitrogen during the preceding 30 years or more; but whilst in 1881 the surface soil of the plots which had grown many leguminous crops showed an average of 0·1058 per cent. of nitrogen, that of the plot which had grown wheat in alternation with fallow, and yielded over so many years only about half as much in crops, contained only 0·0969 per cent. It may be thought that the difference is not great; but a glance at the details which give these means can leave no doubt that it is real; nor can there be any doubt that it is characteristic also. The further results will afford confirmation of this.

The next point to consider is, whether the continued growth of the various leguminous crops has reduced or increased the stock of nitrogen in the surface soils. The foregoing Table shows that in 1881 samples were taken from each of the five differently mineral-manured plots, but in 1882, 1883, and 1885, when samples were taken to considerable depths for the determination of the nitric acid, either plot 4, plot 5, or plot 6 was always selected, as on them the growth was better than on either plot 2 or plot 3.

The following Table (VI.) summarises the percentages of nitrogen in the surface soils (9 inches deep) of the fallow-wheat land, of the *Trifolium repens* plots, of the *Vicia sativa* plots, of the *Melilotus leucantha* plots, and of the *Medicago sativa* plots, in the years as indicated. The figures are as before in all cases the means of 2, 3, or more determinations on each sample. In each case the results given in the first line are the means of determinations made on the individual samples taken from different places on the plot, those in the second line are the means of the determinations made on mixtures of the individual samples, and the general means given in the third line are the means of the results on the individual and on the mixed samples, taken together. It may be further explained that the wheat-fallow samples were taken in 1883 from 4, and in 1885 from 3 places on the plot. The *Trifolium repens* samples were taken in 1882 from 2 places on plot 6, in 1883 from 2 places on plot 4, and in 1885 from 2 places on plot 5. The *Vicia sativa* soils were taken in 1883 from 2 places on plot 4, and from 2 on plot 6. The *Melilotus leucantha* soils were taken in 1882 from 2 places on plot 5, and from 2 on plot 6, and in 1885 from 2 on plot 5. Lastly, the *Medicago sativa* soils were taken in 1885 from 2 places on plot 5. The determinations were, as before, made by the soda-lime method.

TABLE VI.—Percentages of Nitrogen in the fine sifted surface soils (9 inches deep), reckoned as dry at 100° C., at different periods. Samples collected July 26–31, 1882; July 17–26, 1883; July 29–August 14, 1885.

	1882.	1883.	1885.	Mean.
Wheat-fallow land.				
	Per cent.	Per cent.	Per cent.	Per cent.
Means on individual samples	. .	0·1044	0·1006	0·1025
Means on mixtures of individual samples . .	. .	0·1026	0·1035	0·1031
General means	. .	0·1035	0·1021	0·1028
Trifolium repens land.				
Means on individual samples	0·1149	0·1131	0·1269	0·1183
Means on mixtures of individual samples . .	0·1131	0·1125	0·1268	0·1175
General means	0·1140	0·1128	0·1269	0·1179
Vicia sativa land.				
Mean on individual samples	. .	0·1203	. .	0·1203
Mean on mixture of individual samples . . .	. .	0·1178	. .	0·1178
General mean.	. .	0·1191	. .	0·1191
Melilotus leucantha land.				
Means on individual samples.	0·1095	. .	0·1122	0·1109
Means on mixtures of individual samples . .	0·1123	. .	0·1179	0·1151
General means	0·1109	. .	0·1151	0·1130
Medicago sativa land.				
Mean on individual samples	. .	. .	0·1214	0·1214
Mean on mixture of individual samples . . .	. .	. .	0·1224	0·1224
General mean.	. .	. .	0·1219	0·1219

It is seen that even the fallow-wheat soils show slightly higher percentages of nitrogen in the autumns of 1883 and 1885 than in March, 1881; but the later samples were all taken from the end of the field, the samples from which showed somewhat higher percentages than the others even in 1881; and, as the figures show,

the increase was in most cases much greater on the various leguminous plots. Thus, comparing the final means in the foregoing Table with those in Table V. (p. 14), the percentage in the wheat-fallow soil is 0·1028 against 0·0969 in 1881; in the *Trifolium repens* soil it is 0·1179, in the *Vicia sativa* soil 0·1191, in the *Melilotus leucantha* soil 0·1130, and in the *Medicago sativa* soil 0·1219, against a general mean of 0·1058 in 1881.

It should be stated that before taking the samples all above-ground growth is carefully cut off by scissors and removed; and that in the preparation of the soil-samples for analysis, all roots, indeed all visible vegetable débris, is carefully picked out; so that the results only include the nitrogen of that part of the crop-residue which has become thoroughly disintegrated, and may be considered as a proper constituent of the surface mould. It may be further stated that the separated residue from the leguminous crop soils contained more nitrogen than that from the wheat-fallow soil.

Going a little more into detail, it is seen that the *Trifolium repens* soil shows a mean of 0·1140 per cent. nitrogen in 1882, of 0·1128 per cent. in 1883, and of 0·1269 per cent. in 1885. That is to say, the lowest percentage is in 1883 when there had been no growth, when there had been a whole season for the disintegration and nitrification of the residue of the previous year, and when 146 lbs. of nitrogen as nitric acid were found to the depth of 108 inches, and more than four-fifths of it below the surface soil. On the other hand, the percentage is the highest in 1885, when nearly 100 lbs. of nitrogen had recently been removed in the crop, and the crop-residue would be comparatively large.

In the *Melilotus leucantha* soil somewhat more nitrogen was found in 1885, which was the eighth year of continuous crop, than in 1882, which was only the fifth season, but which yielded more than twice the amount of nitrogen in the crop, and left considerably more visible and separated residue in the surface soil.

Lastly, of the *Medicago sativa* soils, we have samples only in the sixth year of the growth, which had rapidly increased to an enormous amount in the fifth year, 1884, and yielded very large, though somewhat less, produce in 1885. Under these circumstances the mean percentage of nitrogen in the surface soil is 0·1219, or higher than in the case of any other plant or year, excepting in the *Trifolium repens* soil of the same year. This is the case notwithstanding that more visible crop-residue had been separated from the *Medicago sativa* soil samples than from any of the others; and in fact about three times as much as from the *Trifolium repens* soils of the same year. Indeed, it was estimated that the separated residue from the *Medicago sativa* soil-samples, the nitrogen in which was determined, represented a removal of about 100 lbs. of nitrogen per acre.

Without relying on the exact figures as representing exact gains or losses of nitrogen by the surface soils, we think it will be granted that the results are too consistent to leave any doubt that by the growth of the leguminous crops the surface soils had gained nitrogen, and that this gain bore some relation to the amount of growth and removal in the crops.

If then the surface soils have gained in nitrogen, it is obvious that they have not been the primary source of the nitrogen taken up by the plants. It must have come either from above or from below the surface soil—from the atmosphere or from the subsoil. The evidence does indeed point to the fact, that much nitric acid results from nitrification of nitrogen accumulated within the surface soil. But as this nitrogen either increases or does not diminish, much of the nitric acid produced must come from some other source than what may be called the original stock of nitrogen of the surface soil itself. Much doubtless comes from the nitrogen of crop-residue, itself derived from the atmosphere or from the subsoil.

Before discussing this subject further it will be well to call attention to another remarkable experiment, showing the amount of nitrogen that may be taken up by one leguminous plant growing on land where another had to a great extent failed.

4. *Experiments on the growth of Red Clover on Bean-exhausted Land.*

The results in question were obtained in a field in which beans had been grown almost continuously for 32 years, but had considerably declined in yield, as the following Table will show :—

TABLE VII.—Quantities of Nitrogen removed per acre per annum, in lbs., in bean-crops, over four periods of 8 years each. Geescroft Field, Rothamsted.

	8 years, 1847–1854.	8 years, 1855–1862.	8 years, 1863–1870.	8 years, 1871–1878.	Average 32 years, 1847–1878.
	lbs.	lbs.	lbs.	lbs.	lbs.
1. Without manure	48·41	25·26	9·12	16·36	24·79
2. With mineral manure containing potash	60·19*	34·25	23·46	26·66	35·36†
3. With mineral manure and nitrogen . .	68·94	36·87	35·05	28·69	42·39

The two upper lines show the amounts of nitrogen removed per acre per annum in the bean crops without any supply of nitrogen by manure. It will be seen that over each period of 8 years, the plot receiving a mineral manure containing potash yielded considerably more nitrogen than the one without any manure. In both cases, however, there was considerable decline from the first period to the last. Further, whilst over the 32 years the unmanured plot yielded an average of 24·8 lbs. of nitrogen per acre per annum, that with the potash manure yielded 35·4 lbs., or nearly one-half more, though without any supply of nitrogen from without. In the third experiment, where besides the potash manure some nitrogen was applied, in the early years as ammonium

* Average of 7 years only, results not available for 1849.

† Average 31 years only.

salts and in the later as sodium nitrate, there is some, but comparatively little increase in the amount of nitrogen in the crop.

In connection with the fact of the gradual decline in yield, it should be explained that owing to failure of the beans there was—in the second period one year of fallow, and one year of wheat; in the third period, one year of fallow; whilst in the fourth period, the first crop failed, and the land was left fallow during the second, third, and sixth seasons. The yields of nitrogen are, however, in each case, averaged for the period of 8 years.

After the 32nd year, 1878, the land was left fallow for between four and five years. Under these circumstances, as will be seen presently, the stock of total nitrogen in the surface soil had become very low, direct determinations of the nitrogen as nitric acid showed that the already existing amount of nitric-nitrogen down to the depth of 72 inches, was extremely small, whilst after several years of fallow there would be a minimum amount of crop-residue remaining for nitrification.

On this land, exhausted for one leguminous crop, barley and clover were sown in the spring of 1883. The clover grew very luxuriantly from the first, much interfering with the growth of the barley.

In our paper in the 'Transactions of the Chemical Society,' for June, 1885, we gave the amount of nitrogen as nitric acid found to the depth of 72 inches on the plot without manure, in that with the mineral manure alone, in that with the mineral and nitrogenous manure, and in that with farm-yard manure. We further estimated that the barley and clover crops would probably remove more than 200 lbs of nitrogen per acre. The amounts have, however, since been determined, and they are as follows :—

TABLE VIII.—Nitrogen removed per acre in the barley and clover crops.

Previous condition of manuring.	1883. Barley and clover.	1884. Clover.	1885. Clover.	Total.
	lbs.	lbs.	lbs.	lbs.
Without manure	45·0	183·2	52·7	280·9
Mineral manure and some nitrogen	57·2	193·1	79·9	330·2
Mineral manure only	59·3	206·4	81·6	347·3

It should be stated that the plots, the yield of nitrogen of which is here given, do not exactly correspond with those as given in the preceding Table, some of the crops being taken together where no difference in the produce was observable. Thus half the plot represented as without manure has been unmanured from the commencement, that is, for nearly 40 years, the other half having received small quantities of nitrogen to 1878 inclusive, but has since been entirely unmanured. Again, the results given in the second line relate to the produce on the plot with the purely mineral manure containing potash, given in Table VII. as No. 2, together with that of the plot No. 3 to

which some ammonium salts, or nitrate had, up to 1878, been applied, but which has received no manure since. The results given in the third line of the above Table (VIII.), relate, however, to a plot which has not received any nitrogenous manure from the commencement, but was not brought into experiment until five years later than the other plots.

Here then, in a field where beans had been grown for many years, had frequently yielded only small crops, and sometimes failed, and the land had then been left fallow for several years, where the surface soil had become very poor in total nitrogen, where both surface and subsoil were very poor in ready formed nitric acid, and where there was a minimum amount of crop-residue near the surface for decomposition and nitrification, there were grown very large crops of red clover containing very large amounts of nitrogen. On a plot where a purely mineral manure, containing potash, had been applied for 27 years up to 1878, but no manure whatever since, 347 lbs. of nitrogen were gathered, almost wholly by the clover. On a plot, on half of which the mineral manure only, and on the other half the same mineral manure with some ammonium salts or nitrate had been applied up to 1878, but nothing since, 330 lbs. of nitrogen were removed in the crops. Lastly, where on half the plot there had been no manure whatever for nearly 40 years, and on the other half ammonium salts or nitrate to 1878, but nothing since, 281 lbs. of nitrogen were yielded in the crops.

It may be said, therefore, that about 300 lbs. of nitrogen had been gathered by the clover growing on a soil upon which beans had yielded smaller and smaller crops, and, in fact, had eventually practically failed, and which was very poor both in total nitrogen near the surface, very poor in ready formed nitric acid to a considerable depth, and very poor in nitrogenous crop-residue for nitrification. If therefore the clover had taken up its nitrogen either wholly or mainly as nitric acid, the supply could not be due to recent crop-residue.

Not only was so much nitrogen removed in the crops, but the surface soil became determinably richer in nitrogen, as the following results will show. The plots are the same as those to which Table VII. refers; and the determinations are those made in samples of surface soil collected in April, 1883, before the sowing of the barley and clover, and in November, 1885, after the removal of the crops.

TABLE IX.—Nitrogen per cent., and per acre, in the surface soils, before and after the growth of the barley and clover. Geescroft Field, Rothamsted.

	Nitrogen in sifted dry soil.				
	Per cent.		Per acre.		
	1883.	1885.	1883.	1885.	1885 + or − 1883.
	p. c.	p. c.	lbs.	lbs.	lbs.
1. Without manure	0·0993	0·1083	2441	2662	+ 221
2. With mineral manure containing potash	0·1087	0·1149	2672	2824	+ 152
3. With mineral manure and nitrogen	0·1163	0·1225	2859	3011	+ 152

Without assuming that the figures represent accurately the amounts of nitrogen accumulated per acre, it cannot be doubted that the surface soils had become considerably richer. If, for the sake of illustration, we assume that 300 lbs. of nitrogen were removed per acre in the crops, and 200 lbs. were accumulated in the surface soil, we have 500 lbs. to account for as gathered by the crops, and chiefly by the clover, within about 2 years.

In our former paper, when we assumed that perhaps 200 lbs. would be removed in the crops, we admitted that there was in the experimental results no conclusive evidence as to the source of so large an amount of nitrogen, but that it must obviously have been derived either from the atmosphere or from the subsoil; and assuming it to be the subsoil rather than the atmosphere, the question arose whether it was taken up as nitric acid, as ammonia, or as organic nitrogen? It was pointed out that as yet no direct proof existed that green-leaved plants did take up the organic nitrogen of the soil as such; and that although there was more evidence from analogy in favour of a nitric acid source than of any other, proof was equally wanting to establish the conclusion that so much nitrogen had been available as nitric acid. The much larger amounts now known to have been gathered by the clover crop, of course renders this explanation still less adequate.

On a review of the whole of the results that have been adduced, it cannot be doubted that nitric acid is an important source of the nitrogen of the Leguminosæ. Indeed, so far as existing experimental evidence goes, that relating to nitric acid carries us quantitatively further than any other line of explanation. But it is obviously quite inadequate to account for the facts of growth, either in the case of the *Medicago sativa* grown on the clover-exhausted land, or in that of the clover on the bean-exhausted land. There is, in fact, nothing in the results relating to the clover experiment to justify the conclusion that there had been such a large production of nitric acid in the subsoil, due to the increased development of the nitrifying organisms under the influence of the leguminous growth and crop-residue, and their

distribution, favoured by the action of the roots, and the increased activity in the interchange of moisture and air which must take place under such circumstances. Nor is it explicable how such large quantities of nitric acid could have been produced as would be required for the rapidly increasing growth of the *Medicago sativa*, and for the large amounts of nitrogen in it, if nitric acid had been the exclusive or even the main source of supply.

5. *Experiments on the Nitrification of the Nitrogen of Subsoils.*

In our paper in the 'Transactions of the Chemical Society' already referred to, we showed, in the case of some prairie land subsoils, that their nitrogen was susceptible of nitrification, and that when, after repeated extraction, the action became very feeble, or ceased, it was renewed on the soils being seeded by 0·1 gram of rich garden mould, which would contain nitrifying organisms. Considering, however, that from the circumstances of the collection and the transmission of those samples, the entire exclusion of comparatively recent organic residue from the upper layers was uncertain, it was decided to experiment in a similar way with some of the Rothamsted raw clay subsoils. Those selected were :—

1. A mixture of samples from the third to the twelfth depths of 9 inches each, that is representing the layer of 90 inches thick from 19 to 108 inches deep, from each of 3 out of the 4 holes from which samples were taken on the wheat-fallow land from July 17–26, 1883.

2. Similar mixtures from the third to the twelfth depths, from the samples taken from July 17–26, 1883, from the *Trifolium repens*, and from each of the two *Vicia sativa* plots, respectively.

3. From holes opened specially for subsoil samples only, one on the wheat-fallow land, on May 7, 1886, and one on April 16, 1886, in a field where red clover had been sown with barley in rotation in the previous spring, but from which no crop had yet been taken.

The first column of the next Table (X.) shows, for each of these samples, the percentage of total nitrogen calculated on the dry sifted soil, as determined by the soda-lime method; and the second column shows the quantity of already existing nitric-nitrogen per million of fine dry soil in each case. The other columns show the amounts of nitric-nitrogen per million dry soil, as determined by SCHLŒSING'S method, in watery extracts made by the aid of the water pump, after successive periods of exposure under suitable conditions as to temperature and moisture. In the case of the wheat-fallow and leguminous crop subsoils, the results relating to which are given in the upper two divisions of the Table, they were each seeded by the addition of 0·1 gram of rich garden soil after the first period of exposure and subsequent extraction, and again by the addition of 0·2 gram after the third period and extraction. These experiments, with the exception of the determinations of the total nitrogen, were made in the Rothamsted Laboratory by Mr. D. A. LOUIS.

In the case of the wheat-fallow, and rotation clover land samples, to which the bottom division of the Table refers, there was a seeding with 0·2 gram of the garden soil after the extraction of the already existing nitric nitrogen, and before the first period of exposure ; and there was again a similar seeding of the " seeded " lots, after the third period of exposure in the case of the wheat-fallow subsoil, and after the fourth period in that of the rotation clover land subsoil. There was also added, in the case of the wheat-fallow subsoil after the third, and in that of the clover subsoil after the fourth period, 0·8 gram of a mixture of 1 part potassium phosphate, 1 part magnesium sulphate, and 5 parts calcium carbonate; the mixture containing as impurity 0·000616 gram nitrogen per gram.

TABLE X.—Results showing the amount of the Nitrification of the nitrogen of subsoils.

	Nitrogen in fine dry soil.	Nitrogen as nitric acid per million fine dry soil.												Total.	
		Original.	Periods of exposure.												
			1st.		2nd.		3rd.		4th.		5th.†				
Wheat-fallow subsoils, collected July 17–26, 1883. Mixture of 3rd–12th depths.															
	Per cent.		Days.		Days.		Days.		Days.		Days.		Days.		
Hole 1	·0492	0·77	28	·106	28	·109	28	·237	28	·247	246	·084	358	·783	
" 2	·0562	0·48	28	·071	28	·208	28	·043	28	·162	246	·225	358	·709	
" 4	·0484	0·54	28	·127	28	·166	28	·066	28	·299	246	·224	358	·882	
Means	·0513	0·60	28	·101	28	·161	28	·115	28	·236	246	·178	358	·791	
Leguminous crop subsoils, collected July 17–26, 1883. Mixture of 3rd–12th depths.															
Trifolium repens. Plot 4	·0592	2·50	28	·132	28	·083	28	·156	28	·303	246	·338	358	1·012	
Vicia sativa. " 4	·0508	1·63	28	·145	28	·015*	28	·185	28	·379	246	·375	358	1·099	
Vicia sativa. " 6	·0360	1·40	28	·108	28	·079	28	·176	28	·276	246	·421	358	1·060	
Means	·0487	1·84	28	·128	28	·059	28	·172	28	·319	246	·378	358	1·056	
Wheat-fallow samples collected May 7, 1886, at a depth of 5 feet. Clover land samples collected April 16, 1886, at 7 feet 4 inches deep.															
Unseeded { Wheat-fallow	·0768	0·720	28	·053	35	·052	49	·107	129†	[·312]‡			241	·524	
Unseeded { Clover land	·0772	0·618	28	·077	35	·060	28	·134	30	·177	139	[·358]	260	·806	
Means . .	·0770	0·669	28	·065	35	·056	39	·121	79	·245					
Seeded { Wheat-fallow	·0768	0·714	28	·140	35	·081	49	·118	129†	[·358]			241	·697	
Seeded { Clover land	·0772	0·626	28	·262	35	·103	28	·437	30	·192	139	[·580]	260	1·574	
Means . .	·0770	0·670	28	·201	35	·092	39	·278	79	·275					

Referring to the results, it is in the first place to be observed that there is very considerable variation in the percentage of total nitrogen in the different subsoils.

* Probably too low.

† The number of days represents the periods of exposure, the periods of activity are uncertain.

‡ The figures given between brackets thus [ ] show the results obtained after the addition of the mineral mixture described in the text.

Indeed, so variable is the amount of nitrogen in samples of our Rothamsted subsoils taken on one and the same plot, dependent on the varying proportions of clay, sand, gravel, chalk, &c., that as we have fully illustrated in former papers, no estimates of the difference in the amounts of total nitrogen, either in the subsoil of the same plot at different periods, or in the subsoils of plots differently manured, or differently cropped, can be relied upon.

Again, as the amount of the "original" or already existing nitric-nitrogen is, as has been very fully shown, greatly dependent on the description, and on the amount of crop that has been grown, and other circumstances, it is not to be expected that it would bear any direct relation to the richness or poverty of the subsoil in total nitrogen.

Referring to the amounts of nitric-nitrogen formed in the different subsoils, and within the different periods of exposure, there is, as is to be expected in the case of an action depending on the development and activity of an organism of the habits, requirements, and mode of action of which we know but little, considerable irregularity, both from period to period with the same sample, and within each period with the different samples.

Confining attention in the first place to the results relating to the wheat-fallow, and the leguminous crop subsoils, recorded in the upper two divisions of the Table, it will be seen that the first, second, third, and fourth periods of exposure each comprised 28 days; whilst the fifth period extended over 246 days, or about 35 weeks, during a considerable portion of which, however, the soils had doubtless become too dry for activity and nitrification. It is probable that a period of 28 days is too short to insure the active development of the organisms, and consequent energetic nitrification. Then again, the extraction of the soils by water under pressure must, it is to be supposed, remove some of the organisms, instead of allowing of their natural multiplication. Comparison of the results from period to period must, therefore, be made with some reservation.

But apart from any irregularities in the case of individual samples, or at individual periods, if we compare for each period the mean results for the three wheat-fallow samples, with the means for the leguminous crop subsoils, it is seen that during four of the five periods, the leguminous crop subsoils show considerably more nitrification than the wheat-fallow ones; and whilst over the total period the wheat-fallow subsoils show an average of 0·791 nitrogen nitrified per million of soil, the leguminous crop subsoils show 1·056 per million.

Again, the figures in the bottom division of the Table, relating to the wheat-fallow and the rotation clover land samples, collected in the spring of 1886, show in all cases comparable as to length of period, more nitrification in the clover land than in the wheat-fallow land subsoil; and this is so both with the unseeded and the seeded samples.

Thus, then, these results with the raw, and mostly clay, Rothamsted subsoils, containing not more than 6 or 8 parts carbon to one of nitrogen, confirm those

previously obtained with the prairie subsoils containing much higher proportion of carbon, in showing that their nitrogen is susceptible to nitrification, provided the organisms, and other essential conditions, are not wanting. These new results also consistently show that there is more active nitrification in the leguminous than in the gramineous crop subsoils. This it must be supposed, is partly due to more active development, and greater distribution, of the organisms themselves, under the influence of the leguminous growth, with its excretions and residue, and partly to the greater actual amount of such easily changeable matters.

The results are also confirmed by those of experiments made in the Rothamsted Laboratory by Mr. WARINGTON, for the most part on quite distinct lines. Thus, in most cases, instead of determining the amount of nitrification taking place in the different subsoils when exposed under suitable conditions, he introduced a portion of the subsoil into a sterilised nitrogenous liquid, and determined whether nitrification took place; the result being taken to show whether or not the organisms were present in the subsoil. In the first experiments, the samples were taken with precautions to avoid any contamination by roots or other organic matter, and the conditions of the sterilised liquids were such as the experience of the time indicated as favourable for nitrification. Upon these results he says ('Chem. Soc. Trans.,' 1884, p. 645): "I am disposed to conclude that in our clay soils the nitrifying organism is not uniformly distributed much below 9 inches from the surface. On much slighter grounds, it may perhaps, be assumed, that the organism is sparsely distributed down to 18 inches, or possibly somewhat further. At depths from 2 feet to 8 feet, there is no trustworthy evidence to show that the clay contains the nitrifying organism. It is however probable that the organism may occur in the natural channels which penetrate the subsoil at a greater depth than in the solid clay."

Subsequently ('Chem. Soc. Trans.,' 1887, p. 118) he experimented with a greater variety of subsoils, taking samples from the wheat-fallow, the *Trifolium repens*, the *Melilotus leucantha*, and the *Medicago sativa* subsoils, when these were exposed for the collection of the samples for the various experiments, our own results relating to which we have given in some detail. Further, some of the samples were now taken in the immediate neighbourhood of lucerne roots, and gypsum was added to the sterilised liquids.

Among the 69 trials made in this new series of experiments, there was no failure to produce nitrification by samples down to 2 feet; there was only one failure out of 11 trials down to 3 feet; but below 3 feet, the failures were more numerous. Taken at 6 feet about half the samples induced nitrification. The order of priority of nitrification diminished from the upper to the lower depths; indicating more sparse occurrence, and more feeble power of development and action.

Examination of the results shows, however, that quite consistently with those which we have described, there was notably more active nitrification with the leguminous than with the gramineous crop subsoils. Thus, compared with the results yielded by

the wheat-fallow subsoils, those by the white clover subsoils were more marked; but this was especially the case with the lucerne plot subsoils, of which more samples, and those from a greater depth, induced nitrification. The same is also observable on a comparison of the results obtained by the samples from the wheat-fallow plot, with those from the rotation red clover plot.

It is then established that the nitrogenous matters of raw clay subsoils are susceptible of nitrification, if the organisms, with the other necessary conditions, are present. It is further indicated, not only that the action is more marked under the influence of leguminous than of gramineous growth and crop-residue, but that the organisms become distributed to a considerable depth even in raw clay subsoils, especially where deep-rooted and free-growing Leguminosæ have grown.

The next question is, how far, in a quantitative sense, do the results aid us in explaining the source of the large amounts of nitrogen taken up by some leguminous crops—as for instance in the case of the *Medicago sativa* grown on the clover-exhausted land, and of the red clover grown on the bean-exhausted land.

In the case of the three leguminous crop subsoils there was, over the total period, only about 1 part of nitrogen nitrified per million of soil; and as the subsoil to the depth experimented on would weigh about 30 million lbs. per acre, the amount of nitrification supposed would represent only about 30 lbs. per acre. Obviously, the conditions of nitrification in which the samples are exposed in the laboratory are very different from those of the subsoil *in situ.* Thus, whilst in the case of the samples in the laboratory, the conditions as to temperature and aëration would be the more favourable, the successive extractions by water under pressure would be liable to remove, not only the mineral matters essential for the development of the organisms, and for the production of nitric acid, but the organisms themselves, whereas in the case of the natural subsoil the tendency would be to multiplication.

Compared with the small amount of nitrification of the nitrogen of the raw clay subsoils shown in the foregoing experiments, in which some of the conditions were more and others less favourable than in the natural subsoil, the following results obtained by Mr. WARINGTON ('Chem. Soc. Trans.,' 1887, pp. 127–9), show how very large may be the amount of nitrification of the nitrogen of such subsoils under more favourable circumstances than those of their natural condition. Thus, he mixed raw clay subsoil with an equal weight of coarsely powdered flint, seeded the mixture with rich garden soil, moistened it, and placed it in a vessel allowing for free access of washed air. Under these conditions he found, when no mineral food was added, in one case 2·4, and in another 3·0 per cent. of the total nitrogen of the subsoil nitrified; and when mineral food was added, he found in one case 4 per cent., and in another 3·6 per cent. of the total nitrogen was nitrified.

Indeed, the greatest difficulty in the way of the supposition that much nitrogen is available to plants by the nitrification of the nitrogen of the subsoil, is, in fact, the want of sufficient aëration. Independently of the greater or less porosity of the sub-

soil itself, and of the channels formed by worms, it is obvious that wherever the roots go, water and its contents can follow; and that, with deep-rooted plants and free growth, there will be active movement of water, and there must be of air also, in the lower layers of the soil. In our former paper we called attention to the fact that in the experiments in 1882, with the greater growth of the *Melilotus*, there remained in the soil less water than in that of the *Trifolium repens* soil, corresponding down to a depth of 54 inches, to a loss of 540 tons per acre, or nearly $5\frac{1}{2}$ inches of rain; and again, in 1883, the *Vicia sativa* soil showed down to 108 inches, less water than the *Trifolium repens* soil, in amount corresponding to between 600 and 700 tons per acre, or to between 6 and 7 inches of rain. Obviously too, the still deeper rooting, and still freer growing *Medicago sativa* would remove still more water.

Although much experiment and much calculation have been devoted by several investigators to the estimation of the degree of aëration of soils and subsoils of different character, the data at command do not justify any very definite conclusions on the subject. The results seem to indicate a probable range of aëration from about 30 to over 50 per cent. of the volume of the soil. But these estimates do not take into account the varying amounts of water in the soil or subsoil. In the case of the subsoils referred to in this paper, each layer of 9 inches in depth retained from about 2 to nearly 4 inches of water, the amount varying very much according to the nature of the subsoil, and especially according to the amount of growth, and the consequent withdrawal of water from below, and its evaporation, chiefly through the plant, but partly also from the surface soil. The amount must obviously also vary very much according to the character of the season.

It may here be observed that supposing the subsoil contained at one time, air equal to one-third of its volume, this would not suffice for the nitrification of as much nitrogen as was taken up for several years in succession by the *Medicago sativa*, or during two years in the case of red clover on the bean-exhausted land. But the nitrogen is not taken up all at once, though most of it will be within a few months of the year, during which period there would be the most active withdrawal of water from below, and evaporation by the plant and surface soil. The replacement of this subsoil water by an equal volume of air would, however, still not suffice. The question obviously arises, how far, or how rapidly, the used up oxygen will be replaced, and on this point there is very little experimental evidence to aid us. We shall refer to the subject again further on.

6. *Can Roots, by virtue of their Acid Sap, attack, and render available, the otherwise insoluble Nitrogen of the Subsoil?*

Thus, then, although the evidence is clear that the nitrogen of raw clay subsoils, which constitutes an enormous store of already combined nitrogen, is susceptible of nitrification, provided the organisms are present, and the supply of oxygen is

sufficient, yet the data at command do not indicate that these conditions could be adequately available in such cases as those of the very large accumulations of nitrogen by the red clover on the bean-exhausted land, and of the increasing and very large accumulations by the *Medicago sativa* for a number of years in succession.

Accordingly, on September 3, 1885, when the holes were open for the soil sampling on the *Medicago sativa* plot, a specimen of the deep, strong, fleshy root of the plant was taken, and on examination it was found that the root-sap was very strongly acid. The roots of three plants were then collected. Of these, No. 1 had four branches, which were respectively—6 feet 4½ inches, 5 feet 10½ inches, 3 feet 6½ inches, and 2 feet 9½ inches in length; No. 2 had two branches—4 feet 10 inches and 2 feet 2 inches in length; and No. 3 had two branches, respectively 3 feet 9 inches and 1 foot 9 inches in length.

The roots were rapidly washed in distilled water, dabbed with clean cloths, weighed, rapidly cut into small pieces, and bruised into a pulp in Wedgwood mortars, with a measured quantity of ammonia-free distilled water. The pulp was then put upon a vacuum filter, and the resulting extract was made up to a given volume with pure distilled water. It was, however, found impossible to get the extract perfectly clear within the limited time it could be exposed to treatment without risk of change, and hence, in these initiative experiments, it was dealt with whilst still somewhat turbid.

The dry matter, ash, and nitrogen were determined in the original root, in the root extract, and in the exhausted root.

The important question was whether the acid root juice would take up nitrogen from a raw clay subsoil such as that from which the *Melilotus leucantha*, the *Medicago sativa*, and the red clover, were supposed to have derived such large quantities in some way. Accordingly, 20 grams of subsoil from the unmanured wheat-fallow plot immediately adjoining the *Melilotus* and *Medicago sativa* plots, were added to a known quantity of the acid root extract in a stoppered bottle, well shaken, and the soil and liquid were left in contact for some weeks, the autumn holidays intervening. It was found, however, on examination, that the extract had lost, and the soil had gained nitrogen, nitrogenous organic matter having been deposited.

In November, 1885, one of the *Medicago sativa* holes was reopened, and fresh quantities of root were collected. These were prepared in substantially the same way as before, but with much greater expedition, more persons being employed. The roots were rapidly passed successively through 4 basins of distilled water, which was renewed as needed.* They were then dabbed with clean dry cloths as before, weighed, cut first into short lengths, and then into very small pieces, the whole being kept in basins covered with glass plates as much as possible to lessen evaporation. The weight was then again taken, a known quantity of pure distilled water added, the whole bruised in Wedgwood mortars, and re-weighed.

* In subsequent experiments it has been found that the wash-water did not become acid during the process.

One weighed portion of the pulp was put on the vacuum filter as before, and another was submitted to dialysis, the diluted pulp being put into a parchment paper sausage dialyser which was then placed in pure distilled water. However, neither the vacuum filtrate nor the dialysate was quite clear. Both these extracts showed a less degree of acidity, and it was evident that the root was now in a very inactive state compared with that of the specimens collected early in September. Various qualitative examinations were made, and it was found that the extracts contained a large amount of nitric acid. It was decided, however, that further detailed investigation of the subject must be postponed until the return of the actively growing period.

It was intended to undertake the subject in the spring and summer of 1886, but owing to the pressure of other work nothing more was accomplished than the comparative testing of the acidity of the sap of the roots of a number of plants representing very various natural families. The matter was, however, again taken up in April, 1887; and, benefiting by previous experience, some advance was made, but still the attempts to entirely free the acid extract from nitrogen were not successful.

It is of interest to observe that the degree of acidity of the sap of the roots collected in April, May, and June, that is during the periods of the most active growth of the season, was considerably higher than in that of the roots collected in September, 1885, after the cutting of the first crop.

The investigation is, however, at present little more than commenced, and any further reference to the results must be postponed to some future occasion.

7. *Action of Dilute Organic Acid Solutions on the Nitrogen of Soils and Subsoils.*

In the autumn of 1885, when it was found necessary to postpone further experiment with the acid root-sap, it was decided in the meantime to examine the action on soils and subsoils, of various organic acids, in solutions of a degree of acidity either approximately the same as that of the *Medicago sativa* root juice, or having a known relation to it. The acids experimented upon were the malic, citric, tartaric, oxalic, acetic, and formic.

In the first experiments, pure water, and dilute solutions of malic, citric, tartaric, oxalic, and acetic acids, were used; each of the acid solutions being of approximately the same degree of acidity as the sap of the *Medicago sativa* roots collected in September, 1885, that is after the removal of the first crop, which represented the greater part of the growth of the season. The subsoil employed was a mixture of the sifted soil of the fifth, sixth, seventh, eighth, ninth, and tenth depths of 9 inches each, from the unmanured wheat-fallow land immediately adjoining the leguminous plots, and contained, as dried at 100° C., 0·047 per cent. of nitrogen.

The mixtures were made in wide-mouthed stoppered bottles, in the proportion of 200 grams of the subsoil to 1000 c.c. of the water or acid solution. After being well

shaken, in the case of the water, the malic acid, and the acetic acid extracts, a given quantity was drawn off at the expiration of one hour. The remainders of these, and the mixtures of the other acid solutions, were frequently shaken, finally left to settle, and after contact for between two and three days the extracts were decanted off, and the soils drained on a vacuum filter. The several extracts being filtered, portions were at once evaporated to dryness on a steam bath, and the nitrogen in them was determined by the soda-lime method. The actual quantity of nitrogen involved in each determination was, however, so small, that recourse was afterwards had to KJELDAHL'S method; and comparative results led to the conclusion that those obtained by it were the more trustworthy. Accordingly, only the general indications obtained by the soda-lime method are here given.

In the case of the water, and of the malic and acetic acid solutions, nitrogen was taken up after 1 hour's contact with the raw clay subsoil; the most being taken up by the malic acid. In each case, after contact for between two and three days, the amounts of nitrogen in the extract were less than after only 1 hour's contact. There had thus obviously been re-precipitation of nitrogenous matter at first taken up, and as the extracts showed scarcely any remaining acidity, the explanation seemed to be that the longer the contact, the more was the acid neutralised by the fixed bases of the subsoil. Of the five organic acid solutions left in contact between two and three days, the malic retained the most nitrogen; next came the acetic and tartaric, then the citric, and lastly the oxalic.

In a second series of experiments, besides the same five acids as before, formic acid was included. The acid solutions were however now twice as strong as those used in the first series. In the case of the malic acid, the periods of contact were 1 hour and 48 hours, and in that of each of the others 1 hour and 24 hours. In each case the acidity of the solution was much reduced by contact with the subsoil, and in each the more the longer the contact. Again the malic acid took up the largest amount of nitrogen; and with the malic, and the formic acids, less was found in the extracts after the longer periods of contact. With the oxalic acid, however, in a striking degree, and less in that of the tartaric, the amount of nitrogen taken up was greater after 24 hours' than after 1 hour's contact, probably owing to the precipitation of the lime in these cases.

It was next decided, with the view of getting larger amounts of nitrogen taken up, to make 3 series of experiments as follows:—

1. With double the quantity of subsoil to a given volume of the acid solution.
2. With double the quantity of acid solution to a given quantity of the subsoil.
3. To add a second quantity of the acid solution to the already once extracted subsoil.

Further, it was decided to experiment with malic acid only; and for comparison with the results on the subsoil, to make parallel experiments with the surface soil of the *Medicago sativa* plot. Lastly, duplicate determinations of the nitrogen in the

extracts were made, one by the soda-lime method, and the other by KJELDAHL'S sulphuric acid method.

In the following Table the results obtained in these experiments by KJELDAHL'S method are given.

TABLE XI.—Showing the amount of the Nitrogen of surface soil and subsoil dissolved by malic acid solution of approximately twice the acidity of the sap of the *Medicago sativa* roots collected in September, 1885.

		Nitrogen dissolved per million soil.	
		After 1 hour's contact.	After 24 hours' contact.
400 grams soil, 1000 c.c. acid solution, each extraction.			
		Per million.	Per million.
Wheat-fallow subsoil	First extraction	2·43	1·82
	Second extraction	2·19	2·19
Lucerne surface soil	First extraction	9·72	8·51
	Second extraction	6·08	7·59
200 grams soil, 1000 c.c. acid solution, each extraction.			
Wheat-fallow subsoil	First extraction	3·28	7·29
	Second extraction	4·03	3·61
Lucerne surface soil	First extraction	8·14	10·81
	Second extraction	4·35	7·31

First as to the experiments the results of which are given in the upper division of the table, in which 400 grams of soil were mixed with 1000 c.c. of acid solution, in each extraction, that is to say, after the removal of the first extract by decantation and the filter pump, a second quantity of the acid solution was added. After 1 hour's contact with the subsoil, the liquid remained only slightly acid, and the amount of nitrogen taken up was very small, representing only 2·43 parts per million of subsoil. After 24 hours' contact the liquid was still less acid, and the amount of nitrogen found in the extract was, calculated per million of subsoil, considerably less than after only 1 hour's contact.

After the addition of a second quantity of the acid solution to the already once extracted subsoil, the liquid remained much more acid than in the case of the first extraction, both after 1 hour's and 24 hours' contact. Still, the amount of nitrogen taken up was very small; being only 2·19 per million soil after 1 hour's, and the same after 24 hours' contact. That is to say, with the greater remaining acidity in the second extraction, there was not less nitrogen taken up after 24 than after 1 hour's contact. It may be observed that, under these conditions, much more total matter, remained dissolved in the second than in the first extract.

Turning now to the parallel results obtained with the lucerne surface soil, which, though poor, still contained about twice and a half as much nitrogen as the subsoil, it is seen that much more nitrogen was found in the extracts than in those from the subsoil. At the same time the liquids after contact showed scarcely a trace of acidity, and they were found to contain much more of other dissolved matters. In the first extraction, after 1 hour 9·72, and after 24 hours 8·51 parts of nitrogen were taken up per million of soil; and in the second extraction 6·08 and 7·59 parts per million. That there was less nitrogen taken up by the second quantity of acid than by the first, is doubtless due to the more readily soluble portion having been already removed. Even in the second extraction of this richer, though still poor, surface soil, about three times as much nitrogen was taken up as from the subsoil.

In the experiments so far considered, nearly the whole of the acid was neutralised in the first extraction of the subsoil, and in both extractions of the surface soil. In the experiments, the results of which are recorded in the lower division of the table, only half the quantity of subsoil or surface soil was mixed with 1000 c.c. of the acid solution; and here, in the case of the subsoil the liquids remained distinctly acid in the first extraction, even after 24 hours' contact, and more strongly acid in the second extraction. In the case of the surface soil, however, in the first extraction the acidity was entirely neutralised, and even in the second extraction nearly so.

The figures show that considerably more nitrogen was taken up, even from the subsoil, when twice the quantity of acid solution was used to a given quantity of it, and when, accordingly, the extracts remained more or less strongly acid. In the first extraction the quantities of nitrogen found in solution were, after 1 hour 3·28, and after 24 hours 7·29, per million soil; that is the more the longer the contact when the liquid remained distinctly acid. In the second extraction, with still greater remaining acidity, the amounts were 4·03 after 1 hour, and only 3·61 after 24 hours. That notwithstanding there was much more remaining acidity, there should be less taken up after 24 hours in the second than in the first extraction, again indicates that a certain quantity of the nitrogen exists in a more readily attackable condition than the remainder. It may be added, that much more mineral matter as well as nitrogen was taken up with the larger proportion of acid solution to a given weight of the subsoil.

With the larger quantity of acid solution to a given weight of the surface soil, much more nitrogen was taken up than under parallel conditions with the subsoil. But, in the first extraction there was little more, and in the second even less, than with twice the quantity of the surface soil to a given quantity of the acid solution. In fact, there was, taking the two extractions together, even less nitrogen taken up with the larger than with the smaller proportion of acid solution to a given weight of soil; but with the larger proportion there was much more mineral matter taken up, whereby the acid would be to a greater degree neutralised. There is, both after 1 hour and after 24 hours, much less nitrogen taken up in the second than in the first

extraction ; again showing that a certain proportion of the nitrogen of the soil is more easily attacked than the remainder.

All the foregoing results illustrating the action of dilute organic acid solutions on the organic nitrogen of soils and subsoils were obtained in 1885, and 1886, by Mr. D. A. LOUIS, and the strengths adopted had reference to the degree of acidity of the sap of the lucerne roots collected in September, 1885, after the main growth of the season was past. But finding the sap so much more strongly acid in April, 1887, that is at the commencement of the active growth of the season, it was decided to experiment with much stronger malic acid solutions.

The following Table gives the results of experiments made by Dr. N. H. J. MILLER ; in which the malic acid solution was of approximately 10 times the acidity of the September, 1885, root-sap, and the mixtures were made in the proportion of 200 grams of the wheat-fallow subsoil to 1000 c.c. of the acid liquid. As before, a portion of the extract was removed after 1 hour's contact, and the remainder after 24 hours'. A second quantity of the acid solution was then added to the already once extracted subsoil, and portions were examined as usual after 1 hour's and after 24 hours' contact.

TABLE XII.—Showing the amount of the Nitrogen of subsoil dissolved by a malic acid solution of a degree of acidity much greater than that of lucerne root-sap.

		Nitrogen dissolved per million soil.	
		After 1 hour's contact.	After 24 hours' contact.
Wheat-fallow subsoil	First extraction . . .	8·16	13·75
	Second extraction . . .	11·71	7·03

Even in the first extraction more than half the acid remained unneutralised, and a larger proportion still in the second extraction. Under these conditions of constant excess of acid, the raw subsoil gives up considerably more nitrogen, though there was, at the same time, much more mineral matter taken up. In the first extraction the amounts of nitrogen taken up per million subsoil were 8·16 parts after 1 hour, and 13·75 parts after 24 hours ; that is more after the longer contact. In the second extraction, however, less remained in solution after 24 hours' than after 1 hour's contact, from which it would appear that nitrogen once taken up had been deposited.

Obviously the conditions of experiments in which an acid solution is agitated with a quantity of soil are not comparable with those of the action of living roots on the soil. The root action would necessarily affect only a very small proportion of the total soil. But the results recorded clearly show that the greater the acidity of the solution, the more nitrogen is taken up, and the question arises, whether the root

action would not effect more resolution on the surfaces actually attacked? Indeed, this must necessarily be the case if such an action is really quantitatively an important source of the nitrogen taken up by deep and strong rooting plants, with strongly acid sap. In illustration of this necessity it may be stated that, even if as much as 20 parts of nitrogen were taken up per million of soil, as was the case in the last-mentioned experiments in the first and second extractions taken together, this would only represent 600 lbs. of nitrogen per acre to the depth examined, namely 108 inches.

Upon the whole, then, the experiments on the action of weak organic acid solutions on raw clay subsoil, or even on a poor surface soil, have not given results from which any very definite conclusions can be drawn, as to the probability that the action of roots on the soil, by virtue of their acid sap, is quantitatively an important source of the nitrogen of plants having an extended development of roots, of which the sap is strongly acid.

That roots do attack certain mineral substances by virtue of their acid sap, was established by SACHS. He sowed seeds in a layer of sand on polished marble, dolomite, and osteolite, and he found that the polished surfaces were, so to speak, corroded, where in contact with the roots. In regard to these results, SACHS says: ('Text-Book of Botany,' 2nd English edition, p. 702) "every root has dissolved at the points of contact a small portion of the mineral by means of the acid water which permeates its outer cell walls." It was to carbonic acid that SACHS attributed the action in these cases; but there seems no reason to suppose that other acids in the root-sap may not exert a similar action. The results which have hitherto been published have however reference only to the taking up of mineral substances from the soil by virtue of such an action; and so far as we are aware the possibility or probability that the nitrogen of the soil or subsoil is so taken up has not been considered.

Provided it were clearly established that the organic nitrogen of the soil, and especially of the subsoil, was rendered soluble by the action of the acid sap of the root, the question would still remain, whether the nitrogenous body is merely dissolved, and taken up by the plant as such, as the evidence at command seems to show is probable in the case of the fungi, or whether the nitrogenous body, after being attacked by the acid, is subjected to further change before entering the plant? To this point we shall recur presently.

Since the experiments at Rothamsted, above referred to, on the character and the action of the root-sap were undertaken, a preliminary notice of experiments on the nitrogenous organic compounds of the soil has been published by Dr. G. LOGES ('Versuchs-Stationen,' vol. 32, p. 201). He found that the hydrochloric acid extracts of soils rich in humus left on evaporation a residue containing a large proportion of the nitrogen of the original soil. In his notice he does not state the strength of the acid used; but from the results it is to be concluded that it was somewhat con-

centrated—indeed of a strength not at all comparable with that of root-sap. Then, the soils, the results relating to which he gives, were extremely rich in nitrogen, and in this respect again bear no comparison with the subsoils from which the lucerne, and other plants experimented upon at Rothamsted, are supposed to have taken up much nitrogen.

Thus, whilst in LOGES' experiments, one of the soils acted upon contained 0·804, and the other 0·367 per cent. of nitrogen, the surface soil of the lucerne plot at Rothamsted which yielded such large amounts of nitrogen in the crops contained little more than 0·120 per cent., and the subsoil from which a large quantity of the nitrogen must have been derived, only from 0·04 to 0·05 per cent. Again of the 0·804 per cent. in the one soil, 0·322, or 40 per cent. of the whole was taken up by the acid, and of the 0·367 per cent. in the other soil, 0·083 or 22·6 per cent. of the whole was taken up. In the richer soil the relation of carbon to nitrogen was as 13·78 to 1, and in the other as 11·74 to 1, whilst the relation of carbon to nitrogen in the hydrochloric extract was in the case of the richer soil 6·8 to 1, and in that of the other about 11 to 1.

By phospho-tungstic acid LOGES obtained precipitates from the acid extracts, which in the case of the richer soil showed only 6·67, and in that of the other 5·74 carbon to 1 of nitrogen. In reference to these results, it may be observed that this is approximately the relation of carbon to nitrogen in the raw clay subsoil at Rothamsted below the depth at which it is materially affected by manuring or cropping.

Thus, we have found the proportion of carbon to nitrogen to be in the surface soil of rich prairie, or permanent grass land, between 13 and 14 to 1; in that of somewhat exhausted arable surface soil, between 10 and 11 to 1; and in raw clay subsoil about 6 to 1.

LOGES states that he has experimented on a great variety of soils, and that he has found in all, without exception, that the hydrochloric acid extract gives the phospho-tungstic precipitate; and he hopes soon to be able to report further on the nature of the highly nitrogenous humic compound obtained. It would thus seem, however, to be an amide or peptone body.

It is of much interest that the nature of the nitrogenous body existing in, or dissolved out of, soils and subsoils should be determined; and to this end it seems desirable to act on soils with stronger acids than those hitherto employed in our own experiments. But results so obtained can obviously have only an indirect bearing on the special question we have in view, namely—whether roots do, by virtue of their acid sap, attack the otherwise insoluble organic nitrogen of the soil and subsoil, and either take it up as such, or bring it into a condition in which it is easily susceptible to further change, and so rendered available as a source of nitrogen to the plant?

Still more recently, MM. BERTHELOT and ANDRÉ ('Compt. Rend.,' vol. 103, 1886, p. 1101,) have published the results of experiments to determine the character of the

insoluble nitrogenous compounds in soils, and of the changes they undergo, when submitted to the action of hydrochloric acid, of various strengths, for shorter or longer periods, and at different temperatures. The soil they employed contained 0·1744 per cent. of nitrogen. It was therefore much richer than our lucerne surface soil, and about four times as rich as our wheat-fallow subsoil. It was shaken in a flask with water, or dilute hydrochloric acid, in the proportion of 500 grams soil to 1000 c.c. liquid. The clear liquid was just neutralised by potash, then made slightly acid, calcined magnesia added, and the ammonia distilled off, collected, and determined. The remaining liquid was then acidified by sulphuric acid, evaporated to dryness, and the nitrogen determined by the soda-lime method, the result indicating the amount of soluble amide. The following is a summary of their results, which we give in parts of nitrogen per million soil, so as to compare with our own :—

	Nitrogen per million soil.					
	As ammonia.			As soluble amide.		
	18 hours, cold.	5 days, cold.	2 hours, at 100° C.	18 hours, cold.	5 days, cold.	2 hours, at 100° C.
1. Pure water	1·7			8·3		
2. 10 c.c. hydrochloric acid to 400 water (= HCl 3·5 gr.)	4·8	8·75	48·8	27·75	30·25	123·6
3. 50 c.c. hydrochloric acid to 400 water (= HCl 17·5 gr.)	14·4	21·4	101·0	60·6	90·5	356 9
4. 100 c.c. hydrochloric acid to 400 water (= HCl 35 gr.)	14·9	30·4	124·1	68·6	96·5	430·3

The authors call attention to the facts, which are clearly brought to view in the above arrangement of their results, that the amounts, both of ammonia, and of soluble amide obtained, increase with the strength of the acid, the time of contact, and the temperature. They point out that these are products of the action of the acid on certain insoluble nitrogenous bodies in the soil, and that the reaction is similar to that which they have observed in the case of urea, asparagin, and oxamid—that is with well defined amides. The insoluble nitrogenous compounds in the soil are in fact, as previously supposed, amide bodies. They also call attention to the fact that when the clear, filtered, acid extract is exactly neutralised by potash, one portion of the amide still remains soluble, whilst another is precipitated, showing that the amides rendered soluble constitute two groups. The fact of such re-precipitation is quite in accordance with the results obtained in our own experiments, in which less nitrogen remained dissolved after 24 than after only 1 hour's contact, when, with the longer contact, the acidity of the extract became neutralised.

A special point of interest in these results, as compared with those of LOGES, is in

the gradation of effect under the varying conditions as to strength, time, and temperature, and in the evidence as to the proportion of the total nitrogen taken up, which is found as ammonia. The proportion of ammonia-nitrogen to amide-nitrogen ranges from about 1 to 5 to 1 to 3, dependent on the conditions. According to the figures, it would seem that the proportion of the total nitrogen dissolved which is determined as ammonia is the greater, the stronger the acid and the longer the contact.

As in LOGES' experiments, so in these of MM. BERTHELOT and ANDRÉ, the strength of acid used was in all cases much greater than that in any of the Rothamsted experiments, and very much greater than is likely to occur in any root-sap. Indeed, not only was the soil operated upon by MM. BERTHELOT and ANDRÉ about four times as rich in nitrogen as the Rothamsted subsoils, but in the most extreme case, that with the strongest acid, and a temperature of 100° C., nearly one-third of the total nitrogen of the soil was dissolved. Hence, although their results are of great interest as indicating the character of the nitrogenous bodies existing in soils, and of the changes to which they are subject when acted upon by acids, they, like those of LOGES, have only an indirect bearing on the question whether by the action of the organic acids of the root-sap, the insoluble organic nitrogen of the soil, and especially of the subsoil, is rendered available as a source of nitrogen to the plant. Supposing this to be the case, as already said, the further question still remains—whether the dissolved amide is taken up as such, or whether it is subject to further change within the soil before serving as food for the plant?

The fact that the formation of ammonia seems to be an essential element in the reaction, points to the conclusion that at any rate part of the nitrogen liberated from the insoluble condition is available in other forms than as soluble or dissolved amide; and, as our experiments show that nitric acid, as well as ammonia, is a constituent of the root-sap, the question arises—whether the liberated ammonia is oxidated into nitric acid before being taken up? Then, again, is the soluble amide taken up as such, or subjected to further change, perhaps first yielding ammonia, and this again nitric acid?

On this supposition we are met again with the difficulty as to the sufficient aëration of the subsoil for such a purpose. It has already been pointed out, that such evidence as exists on the subject clearly shows that the amount of oxygen within the soil at any one time is totally inadequate for the nitrification of the amount of nitrogen taken up by some plants within the season; whilst the replacement by air of the water evaporated would still be quite insufficient. With what rapidity, or to what extent, the oxygen of the subsoil air would be replenished from above as it is used up, there is no experimental evidence at command to show. But whether this would take place adequately or not, it must be supposed that it would occur to some extent.

Turning to the alternative aspect, inasmuch as the insoluble organic nitrogen of the soil exists in the condition of amide bodies, and the chief first product of the action of

acids is soluble amide, it is of interest to consider, whether plants can take up such bodies and assimilate their nitrogen? There can be little doubt that fungi can utilise both the organic carbon and the organic nitrogen of the soil, though they seem to develop the more freely when the humic matters have not undergone the final stages of change by which the compound of so low a proportion of carbon to nitrogen as is found in raw subsoils, has been produced.

8. *Evidence as to whether Chlorophyllous Plants can take up Complex Nitrogenous Bodies, and Assimilate their Nitrogen.*

The first direct experiments to determine whether green leaved plants can take up organic nitrogen were made in 1857 by Dr. (now Sir CHARLES) CAMERON. He experimented with barley, in an artificial soil, and found that when urea was the only soil-source of nitrogen, the plants grew luxuriantly, and took up much of the nitrogen so supplied. Ammonia was not detected in the soil. Hence he concluded that the urea was taken up by the plant as such. No reference is made to nitric acid, and in the absence of evidence to the contrary, it is possible that nitrates were formed, and served as the source of nitrogen to the plants.

In 1861, Professor S. W. JOHNSON, of Yale, made experiments with maize in an artificial soil. A given quantity of nitrogen was supplied, in one case as uric acid, in a second as hippuric acid, and in a third as guanine. Compared with results in a control experiment without nitrogenous supply, the growth was very greatly increased; and there was no doubt that the substances named had supplied nitrogen to the plants. Professor JOHNSON states that the conditions of the experiments were not such as to demonstrate that the nitrogenous organic bodies entered the plants without previous decomposition, but from the results of CAMERON, and of HAMPE, he concludes that this was the case.

In 1865, 1866, and 1867, Dr. W. HAMPE ('Versuchs-Stationen,' vol. 7, p. 308, vol. 8, p. 225, vol. 9, p. 49, and vol. 10, p. 175) made several series of experiments, all by the water-culture method. Maize was the plant selected, and the sources of nitrogen supply were—urea, ammonium phosphate, uric acid, hippuric acid, and glycocoll.

At first the experiments with urea were not very successful, apparently owing to an unfavourable condition of the solutions as to mineral supply. Afterwards the plants produced were nearly as good as those grown in a garden; ripe seeds being formed, which, when sown, germinated.

Urea was found in the leaves, stems, and roots. Small quantities of ammonia were sometimes found in the solutions, but only when there was some decomposition of the roots or their excretions, and such formation of ammonia was the most prominent after the blooming. To obviate such formation as far as possible, the solutions were in the later experiments frequently renewed. In corresponding solutions without plants,

ammonia was only found twice throughout the summer. Neither nitrates nor nitrites were ever found in the solutions.

HAMPE concluded that the urea was taken up by the plants as such, and that it served as a source of nitrogen to them. This result he considered not inconsistent with the view that plants, other than fungi, cannot utilise products of the plant itself, such as the alkaloids, which they can no more assimilate as a source of nitrogen, than they can sugar as a source of carbon. Urea being, on the other hand, a product of the degradation of animal substance, there seemed no reason why it should not serve as a source of nitrogen to plants.

In his experiments with ammonia HAMPE used the phosphate; and small and large maize were the plants selected. In their early stages the plants seemed to suffer rather than to benefit by the ammoniacal supply; but eventually they gave good growth, and produced ripe seeds, which on being re-sown germinated.

In the experiments with uric acid the same descriptions of maize were employed. The solutions were frequently renewed, and in those which were removed ammonia was always found, but not uric acid. But even in solutions without a plant the uric acid rapidly decomposed. From the results it was concluded that the uric acid had served as a source of nitrogen to the plants; though probably not directly, but by its products of decomposition.

In the case of hippuric acid, applied as hippurate of potash, and to the same descriptions of plant, the growth was somewhat dwarf; but seeds, which were found to germinate, were produced. Benzoic acid was always found in the solutions after vegetation, and also in corresponding solutions without a plant. In both cases a mould formed on the surface, but not in the body of the liquid. The question arose whether the benzoic acid was only formed in the solution under the influence of the mould acting as a ferment, or whether in part in the plant itself, glycocoll being at the same time produced, and serving as the nitrogenous supply? If the latter were the case, the action would be the convrese of that which takes place in the animal, when benzoic acid unites with glycocoll, forming hippuric acid, which is eliminated.

Direct experiments were also made with glycocoll itself. With it, the plants were better than in any of the other experiments. At each renewal of the solution, the old liquid was examined both for glycocoll and for ammonia. Glycocoll was always found, but ammonia only in very small quantity, and its occurrence was apparently connected with decomposition of plant-substance. HAMPE concluded that glycocoll was as available as nitrogenous food to plants as nitric acid.

In 1868 Dr. P. WAGNER ('Versuchs-Stationen,' vol. 11, p. 287) made experiments in continuation of those by HAMPE above described. He repeated, with some modifications, the experiments with ammonium salts, hippuric acid, and glycin, and also experimented with kreatin.

With ammonium phosphate good growth was obtained. Neither nitrate nor nitrite

could be found in the plant, and it was concluded that, as in HAMPE's experiments, the ammonia had served as a supply of nitrogen.

When ammonium carbonate was used, nitric acid was found both in the solution and in the plant; and it was concluded that the ammonia had not served directly as a supply of nitrogen.

In HAMPE's experiments with hippuric acid, it was proved that it served as a supply of nitrogen to the plant; but as benzoic acid was found not only in the vegetation solution, but in a corresponding solution without a plant, and there was, in both cases, fungoid growth on the surface, it was uncertain whether the breaking up of the hippuric acid had taken place only externally to the plant, under the influence of the fungus acting as a ferment, or also within the plant itself, benzoic acid being excreted. By excluding the access of the air, and by frequently passing carbonic acid through the solutions, the formation of the fungus was prevented. Benzoic acid was, however, still found in the plant-solution, but not in the solution without a plant.

WAGNER concluded that hippuric acid was broken up within the plant itself, benzoic acid being excreted, and that it also suffered decomposition in the solution by the agency of the fungus.

HAMPE had obtained very good growth with glycin, but WAGNER thought it desirable to prevent the formation of the mould on the surface of the solution. This he succeeded in doing by frequently passing carbonic acid through it, and glycin was then easily detected in it. Ammonia was only found when there was some decay of the roots. WAGNER concluded that the glycin had been taken up by the plant as such, and had contributed nitrogen to it.

Kreatin was used as being closely allied to urea, which had been proved to serve as a supply of nitrogen to plants. For some time neither mould, nor ammonia, nor smell, was developed in the vegetation-solution; when they were, it was renewed; and some ammonia again appeared when the roots showed signs of decay. WAGNER could not detect kreatin in the plant, as HAMPE had urea. But from its constant presence in the solution, and the very little development of ammonia, he concluded that it served as nitrogenous food to the plant as did urea.

WAGNER considered it established that the higher plants can obtain nitrogen from complex organic bodies as well as from ammonia and nitrous and nitric acids, and that thus the doctrine of the nutrition of plants was much extended. He did not suppose that such a source was essential, and whether in the case of plants growing in soil such substances would serve as a direct supply would depend on the length of time they could remain in such a medium in an undecomposed condition.

The last experiments of this description to notice are those of W. WOLFF with tyrosin ('Versuchs-Stationen,' vol. 10, p. 13). He had formerly experimented with KNOP, on leucine, tyrosin, and glycocoll; and he now repeated the experiment with tyrosin, to determine whether it served directly, or only by its products of decompo-

sition, as the source of nitrogen. To this end the water-culture method was adopted, and rye was the plant selected.

According to the report, the vegetation went on for more than a year—430 days! The amount of the dry substance produced was 365 times that of the seed sown; but no seed was developed. Neither ammonia nor nitric acid was found in the solutions. But, on boiling, a small quantity of an organic body was deposited. From 4·5 grams tyrosin the vegetation acquired 0·18 nitrogen, corresponding to 2·3 tyrosin. No tyrosin was found in the extract of the stems and leaves, but traces were detected in that of the roots.

W. Wolff concluded that tyrosin suffered change as soon as it entered the plant, and that thus the action differed from that found by Hampe in the case of urea. He considered that the tyrosin was, at any rate in part, transformed in the solution, under the influence of the roots; but that ammonia was not one of the products of the change. If the tyrosin were taken up at all as such by the roots, it did not pass unchanged to the upper organs; but when its nitrogen, in whatever form, was assimilated by the plant, it was distributed through the various organs, as in the case of land plants growing under natural conditions.

From the various results above quoted it seems at any rate very probable, if not absolutely demonstrated, that green-leaved plants can take up soluble complex organic bodies, and assimilate their nitrogen, when they are presented to them under such conditions as in water-culture experiments. Even under such conditions, however, if the nitrogenous substance supplied was readily subject to change in the solution itself, it was doubtful whether it was taken up as such, or only after first undergoing change; and it is pretty certain that such substances supplied to the soil, would either in great part or entirely suffer change before being taken up by the plant.

The probability that the higher plants can, under any circumstances, take up complex nitrogenous bodies, and appropriate their nitrogen, is of considerable interest from a theoretical point of view. But under the ordinary condition of the growth of plants in soil, such substances will seldom if ever be available to them, excepting it may be under the influence of the action of the root-sap in rendering soluble the nitrogenous compounds of the soil and subsoil, which exist in them in an insoluble condition.

It will be of interest next to consider what evidence exists as to other modes in which green-leaved plants may acquire nutriment from compounds existing in an insoluble condition in the soil and subsoil.

Dr. Frank has observed that the feeding roots of certain trees are covered with a fungus, the threads of which force themselves between the epidermal cells into the root itself, investing the cell, but not penetrating the fibro-vascular tissue. In such cases the root itself has no hairs; but there were similar bodies external to the fungus-mantle, which were prolonged into threads among the particles of soil. The

fungus-mantle dies off on the older portions of the root, and its extension is confined to the younger roots—those which are active in the acquirement of nutriment.

This fungus development was always observed in the case of teaks, beeches, hornbeams, and hazels:—in seedlings of 1, 2, or 3 years old, and in trees more than a century old. It was, however, not found on the roots of the associated woodland plants, even when these were growing close to a tuft of the mycorhiza. Nor was it found on the roots of maples, elms, alders, birches, mulberry, buckthorn, planes, walnut, apple, service-tree, hawthorn, cherry, cornel ash, syringa, or elder, &c. Thus, the majority of woodland trees appear to be free, and the occurrence seemed to be almost limited to the Cupuliferæ; though outside of this family the development has nevertheless been observed, as on willows, and some conifers; and it is supposed probable that it may be found to be more general as investigation extends.

In the case of the Cupuliferæ the occurrence seems to be universal. It has been observed in the most widely distant localities, at very different altitudes, in very different aspects, in soils of the most varied geological character, and with very varied amounts of humus, with great variation in the associated herbage, and even in a flower-pot. The growth is perhaps the most luxuriant on chalk soils. It is also the more developed in the first 2 inches, or the richer-in-humus layer of the soil.

The occurrence of a fungus on the roots of certain trees has indeed been recorded before. It has sometimes been considered to be connected with a diseased condition, though it has also been noticed on healthy trees. The observations have, however, not before been generalised.

FRANK considers that the conditions are those of true symbiosis. He in fact concluded that the chlorophyllous tree acquires the carbon, and the fungus the water and the mineral matters, that is the soil nutriment.

FRANK did not refer to nitrogen. But there is no reason to suppose that the fungus could not, as do the fungi in the case of fairy rings for example, avail itself of the organic nitrogen of the soil.

Here then we have a mode of accumulation of soil nutriment by some green-leaved plants, which so far allies them very closely to fungi themselves. Indeed, it is by an action on the soil which characterises non-chlorophyllous plants, and by virtue of which they are enabled to take up nutriment not available to most green-leaved plants, that the chlorophyllous plant itself acquires its soil-supplies of nutriment. Under such circumstances, it can indeed readily be supposed that the tree may acquire not only water and mineral matter, but organic nitrogen from the soil, and if so probably organic carbon also. In reference to this point, it has already been stated that, from the evidence so far at command, it was concluded that the action is the most marked in the surface layers of the soil rich in humus.

So far as this is the case, it is obvious that such an action of fungi on the soil does not aid us in the explanation of the acquirement of nitrogen from raw clay subsoil by the deep and strong rooted Leguminosæ. Further, it is distinctly stated that the

fungus development in question has not been observed on the roots of any herbaceous plants. It is nevertheless a point of interest, should it be established, that by special means, in special cases, the organic nitrogen of the soil may serve as a supply of nitrogen to chlorophyllous plants. To this point further reference will be made in the course of the discussions upon which we have now to enter.

PART II.

Recent Results and Conclusions of others, relating to the Fixation of Free Nitrogen.

In our introductory remarks it was stated that the object of the present paper was not only to discuss our own results bearing on various aspects of the question of the sources of the nitrogen of vegetation, but to consider the recent results and conclusions of others, and to endeavour to determine how far the evidence yet available is conclusive on the subject. And, as there can be no doubt that the Memoirs of M. Berthelot have materially influenced the course of inquiry in recent years, it will be well to commence with a statement and discussion of his results and conclusions.

1. *The Experiments of* M. Berthelot.

It was, we believe in 1876, that M. Berthelot first called in question the legitimacy of the conclusion that plants do not assimilate the free nitrogen of the air, when drawn from the results of experiments in which the plants were so enclosed as to exclude the possibility of electrical action. More recently he has objected to experiments so conducted with sterilised materials, on the ground that, under such conditions, the presence, development, and action of micro-organisms are excluded. Such objections, if valid, of course put out of court the results and conclusions of Boussingault, ourselves, and others, from experiments so conducted. They at the same time, obviously suggest, though it is true they do not actually necessitate, the adoption of less exact methods of experimenting—methods in which the soils, or plants, or both, are almost unavoidably exposed to accidental sources of unknown amounts of combined nitrogen, and in which the personal equation becomes a very prominent element. At any rate, since the announcement and acceptance of M. Berthelot's objections, numerous experiments have been made without the enclosure of the plants; and results have been obtained showing very various and, in some cases, very large gains of nitrogen, assumed to be due to the fixation of the free nitrogen of the air in some way.

In 1876 ('Compt. Rend.,' vol. 72, pp. 1283–5) M. Berthelot published the results of experiments in which he found that free nitrogen was fixed by various organic compounds, under the influence of the silent electric discharge, at the ordinary

temperature. Such fixation was determined in the cases of benzene, oil of turpentine, marsh gas, and acetylene. In each case a solid nitrogenous body was obtained, from which ammonia was evolved on strongly heating. The electricity was developed by a large Ruhmkorff coil, so that the conditions were comparable with those between the clouds and the ground during a thunder-storm, and the application of the results to vegetation was legitimate for such conditions. He suggests that similar reactions probably take place in the air during storms, and when the air is charged with electricity, organic matters absorbing nitrogen and oxygen.

Again in 1876 ('Compt. Rend.,' vol. 82, pp. 1357–1360), he recurs to the subject. He says that under the influence of the silent electric discharge, nitrogen, whether pure or mixed with oxygen, is fixed by moist filter paper, and by dextrine, to a degree that is very noticeable within a few hours. Neither ammonia, nor any nitrogen acid is a product of the reaction; and thus the fixation may take place in nature without the preliminary formation of ozone, ammonia, or nitrogen acids.

Subsequently ('Compt. Rend.,' vol. 83, 1876, pp. 677–682), he used currents of much weaker tension, more comparable with those incessantly occurring in the air, and the substances experimented upon were moistened filter paper, and a strong solution of dextrine. The tension would correspond to that between the ground and a layer of air two metres above it. The experiments lasted about two months, during which, however, the tension varied considerably, but averaged $3\frac{1}{2}$ elements Daniell. In all cases nitrogen was fixed by the organic substance, forming a nitrogenous compound from which ammonia was evolved by soda-lime.

In 1877 ('Compt. Rend.,' vol. 85, p. 173) he gives further results of the same kind. In experiments in which the difference of electrical potential was not greater than that frequently existing between strata of the atmosphere not far from the ground, he found that filter paper moistened with water and containing 0·010 per cent. of nitrogen, after a month contained 0·045 per cent., whilst similar paper moistened with a solution of dextrine had its percentage of nitrogen raised from 0·012 to 0·192. He considered that his experiments indicated the true explanation of the fixation of nitrogen in nature. The gains are in amount such as would explain how crops acquire the amounts of nitrogen which he considers they must derive from the atmosphere.

In the autumn of 1885 ('Compt. Rend.,' vol. 101, pp. 775–784) M. BERTHELOT gave the results of experiments on the fixation of atmospheric nitrogen by certain argillaceous earths. He refers to his experiments which established the fact that nitrogen was fixed in some of the immediate principles of plants by the agency of electricity of such feeble tension as is operative all over the globe. He has now to call attention to another mode in which free nitrogen is brought into combination—namely by argillaceous soils under the influence of micro-organisms.

He experimented with two argillaceous sands, and two pure clays—crude kaolins. Some of the experiments were commenced in 1884, but others not until April, 1885;

and for all of these comparative results are given for the period from April 30 to October 10, 1885. It may be mentioned that at the commencement of this period the initial amounts of nitrogen in these materials were very much lower than in any cultivated soils, being respectively 0·0091, 0·01119, 0·0021, and 0·01065 per cent.

Each of these descriptions of soil was exposed under the following conditions :—

1. From 50 to 60 kilog., in open glazed pots, in a closed chamber free from emanations.
2. From 0·08 to 0·10 mm. depth of soil, in open pots, on a trestle 0·7 metre above the ground, in a meadow, with a roof protecting from vertical, but not from oblique rain, or from free air.
3. In similar pots, uncovered, placed on a plank on a tower 29 metres high.
4. 1 kilog. soil, placed in a 4-litre flask, moistened, and closed with a ground stopper; one set being exposed to diffused day light, and a duplicate set kept in a closed cupboard.
5. 1 kilog. of soil put into a 4-litre balloon, heated at 100° C. for 2 hours, steam passed through for 5 minutes, and cooled in filtered air previously heated to 130° C.; then closed and exposed from July 10 to October 6, 1885.

The following tabular statement, summarises the results obtained in the first, second, third, and fourth series of experiments. The upper division shows the actual percentages of nitrogen found, before and after exposure, and the lower division, the gains in the percentage of nitrogen. We give the results in percentages, instead of in parts per kilogram, to compare the better with the figures given relating to our own experiments.

	Initial.	In closed chamber.	In meadow.	On tower.	In closed flasks in light.
		Nitrogen found—per cent.			
Yellow argillaceous sand I.	0·00910	0·01179	0·00983	..	0·01289
,, ,, ,, II.	0·01119	0·01639	0·01295	0·01396	0·01503
White clay	0·00210	0·00407	0·00353	0·00557	0·00494
Crude kaolin	0·01065	..	0·01144	0·01497	0·01236
		Gain of nitrogen—per cent.			
Yellow argillaceous sand I.		0·00269	0·00073	..	0·00379
,, ,, ,, II.		0·00520	0·00176	0·00277	0·00384
White clay		0·00197	0·00143	0·00347	0·00284
Crude kaolin		..	0·00079	0·00432	0·00171

Thus, although the actual amounts of gain are small, there is in every case some ain. Determinations of nitric acid and ammonia showed that the gains were not

correlative with the amounts of either. Further, calculations showed that the amounts far exceeded those which could be due to ammonia in the air, or to ammonia and nitric acid in the rain; whilst the gains in the closed flasks showed that they could not be due to combined nitrogen from the air or rain.

The author considers the results establish the fact that there is gain of nitrogen quite independently of any absorption of combined nitrogen.

From the evidence so far it might be concluded that the gains in the meadow and on the tower were due to electrical action; but the fifth series of experiments, in which the soils were sterilised by heat, and then left in the balloons from July 10 to October 6, 1885, indicate another influence. In the case of each of the four soils so sterilised, and afterwards exposed, there was, instead of any gain, a slight loss of nitrogen, which was attributed to the heating at the commencement. The cause of the fixation of nitrogen had at the same time been destroyed; nor did the soils recover the power of fixing nitrogen, either by exposure to the air of the chamber, or when a small quantity of the unsterilised soil was added.

It was concluded that there was a fixation of free nitrogen due to living organisms. It was shown that the action was not manifested during the winter, that it was the most effective during the periods of active vegetation, and that it was exercised in closed vessels as well as in the free air.

M. BERTHELOT estimated that the gains corresponded to gains of nitrogen per hectare of 20 kilog. by sand No. 1, of 16 and 25 kilog. by sand No. 2, and of 32 kilog. by the kaolin No. 3. These estimates are, however, said to be much too low, as they are on the assumption of only 0·08 or 0·10 m. depth of soil, whilst the action extends much deeper. He compares these amounts with 17 kilog. the amount of combined nitrogen in the rain, &c., at Montsouris in 1883; and with 8 kilog., the amount formerly estimated as annually so coming down at Rothamsted; which, however, more recently we have estimated at less than this. On the other hand, taking the amount of nitrogen removed in a crop of hay at from 50 to 60 kilog. per hectare (= 45 to 54 lbs. per acre), he estimates that the loss to the soil will be from 40 to 50 kilog. per hectare (=36 to 45 lbs. per acre). Hence, if it were not for compensation by fixation of free nitrogen, the soil would gradually become exhausted. He considers that the results bring to view not only one of the methods by which fertility is maintained, but that they also show how argillaceous soils, which are almost sterile when first brought into contact with the air, come to yield more and more flourishing crops, and in time become vegetable moulds.

Quite recently, March, 1887 ('Compt. Rend.,' vol. 104, pp. 625 *et seq.*), M. BERTHELOT has published the results of experiments on the fixation of free nitrogen by vegetable mould supporting vegetation. The experiments were commenced in May, and concluded in November, 1886. He determined the nitrogen by the soda-lime method, and also as nitric acid, in the soil before and after the growth; also in the initial plants (*Amaranthus pyramidalis*), and in the final products. He also determined

the amount of atmospheric ammonia absorbed by sulphuric acid, and the amount of combined nitrogen in the rain; and finally the amount of combined nitrogen in the drainage waters.

The following is a summary of the amounts of nitrogen involved (in grams):—

Initial—In soil 54·09, in rain 0·053, in ammonia of air 0·048, in plants 0·35, total	= 54·541
Final—In soil 56·54, in drainage 0·403, in plants 2·235, total .	= 59·178
Gain	= 4·637

M. BERTHELOT points out that the gain of nitrogen is nearly equally divided between the soil and the plant, the latter having taken it up from the soil, which he considers is the true source of the gain. He compares the results with those formerly obtained without vegetation thus—

Gains with vegetation	4·64	and	7·58	grams.
Gains without vegetation	12·70	and	23·15	grams.

He assumes that there is with the higher plants, as with animals, a constant loss of nitrogen. He admits however that more evidence is needed absolutely to demonstrate that the plants themselves do not fix, and that they do set free, nitrogen. But he considers it proved by his experiments that vegetable soil does fix free nitrogen; and he thinks it probable that this is the chief source of the gain by the higher plants. Thus, it can be understood how concentrated production exhausts faster than the natural actions restore fertility; whilst in natural vegetation, on the other hand, the fixing of nitrogen may exceed the liberation, and accumulation may thus take place.

Reviewing the whole of these results and conclusions of M. BERTHELOT, it is in the first place to be observed that whilst the results obtained under the influence of the silent discharge in bringing nitrogen into combination with certain vegetable principles, owed their special interest to the inference that thus free nitrogen might be brought into combination within the plant, he now considers it at least doubtful, whether the higher plants do bring free nitrogen into combination at all, and that probably the gain of nitrogen is by the soil, and not by the plant.

Obviously, if there are organic compounds existing within the soil which have the power of bringing free nitrogen into combination under the influence of electricity of feeble tension, such as occurs in the atmosphere, the soil and not the plant may be the source, and yet the agent be the feeble electric current. So far, however, as it is assumed that nitrogen is so brought into combination in the atmosphere itself, the resulting compound or compounds will be found in the air, and in the aqueous depositions from it; and the extent, or rather the limit, of the amount of combined nitrogen so available over a given area, in Europe at any rate, is pretty well known.

As to the results obtained with soils, with and without vegetation, it must be admitted that M. BERTHELOT has carefully considered, and endeavoured to estimate, all other apparent sources than free nitrogen. At the same time, the conditions of risk and exposure to accidental sources of gain in the experiments in the chamber, in the meadow, and on the tower, are such that the results could not of themselves be accepted as at all conclusive. To the distinct gains observed in the experiments in closed vessels no such objection can however be raised ; whilst the negative results in the sterilised soils constitute another element in favour of the conclusion at which M. BERTHELOT has arrived.

It is, however, one thing to accept experimental results on the authority of M. BERTHELOT, and another to adopt his arguments and conclusions in the application of them to the conditions of practical agriculture. To avoid repetition, however, further reference to this part of the subject must be postponed until the results and conclusions of other experimenters have been considered; for, to a great extent, the same facts and arguments are applicable in reference to them, as to M. BERTHELOT'S results and conclusions.

2. *The Experiments of* M. P. P. DEHÉRAIN.*

The plan and methods of M. DEHÉRAIN'S experiments to determine the losses or gains of combined nitrogen were totally different from those adopted by M. BERTHELOT. They were indeed much on the lines of some of the Rothamsted investigations. He sought to determine the actual losses or gains in the field, under the influence of different manures, of different crops, and of different modes of cultivation. His experiments were made on the farm of the Agricultural School, at Grignon, near Paris, and extended from 1875 to 1885 inclusive. The land had been in lucerne for 5 years, 1870–1874. Four plots were then devoted to each experimental crop as under :—

No. 1 received farm-yard manure, No. 2 nitrate of soda, No. 3 ammonium sulphate, and No. 4 was left unmanured. Each of the manures was applied 3 years in succession, and then the crops were grown for four years more without further manuring.

On one of the sets of four differently manured plots, green maize was grown. On a second set potatoes were grown during the 3 years of manuring, and for two years afterwards, and then wheat for the two remaining years. On the third set beet was grown for 3 years, green maize for 1 year, and then sainfoin for 5 years, and mixed grasses for 2 years, to 1885 inclusive.

The nitrogen was determined in the soil, before the commencement of the experiments in 1875, in 1878 after the three years of manuring and cropping, in 1881 after 4 years cropping without further manuring, and in case of the sainfoin followed by mixed grasses, in 1885 also. Lastly the nitrogen was estimated in the crops. From

* 'Annales Agronomiques,' vol. 8, p. 321, vol. 12, p. 17, and vol. 12, p. 97.

these data, the losses or gains of nitrogen by the soil, during the different periods under the influence of the different manures and crops, were calculated.

M. DEHÉRAIN further gives the results of numerous determinations of carbon in the soils, and shows that with a loss of nitrogen there is also a loss of carbon; and that where in the case of the growth of sainfoin, the nitrogen in the surface soil increased, there was not a reduction in the carbon.

In his first paper M. DEHÉRAIN summarises his conclusions as follows:—

1. The soil of each experimental plot lost nitrogen from 1875 to 1878 and 1879, when it had grown green maize or potatoes; it also lost when beet was grown.

2. The loss much exceeded the amount due to the removal of the crop.

3. The loss was very sensible even when the soil received abundance of manure, and it continued from 1878 or 1879 to 1881, when the soil grew maize, or potatoes followed by wheat.

4. When, from 1879 to 1881, sainfoin was substituted for beet, not only was loss no longer manifested, but the nitrogen of the soil augmented, and at the same time abundant crops of sainfoin were obtained, which contained large quantities of nitrogen.

5. This nitrogen has not come from the deeper layers of the soil, for these showed an equal, or even rather greater richness in 1881 than in 1879.

With regard to the actual amounts of loss or gain of nitrogen found in M. DEHÉRAIN'S experiments, the losses especially are extremely large, as the following results will show:—

When farm-yard manure was applied, in very heavy dressings for three years in succession, in amount estimated to supply 400 kilog. nitrogen per hectare per annum (= 357 lbs. per acre per annum), there was, when green maize was grown, a loss of nitrogen by the soil, besides that removed in the crops, amounting to 288 kilog. per hectare (= 257 lbs. per acre) per annum, over the 3 years of the application; when potatoes were grown there was a loss of 242 kilog. per hectare (= 216 lbs. per acre) per annum; and when, with the same manuring for 3 years, beet was grown for 3 years and maize for one year, there was an average annual loss over the 4 years of 679 kilog. nitrogen per hectare (= 606 lbs. per acre).

When nitrate of soda, supplying 192 kilog. nitrogen per hectare (= 171 lbs. per acre) per annum, was applied, the annual loss of nitrogen was, when maize fodder was grown, 401 kilog. per hectare (= 359 lbs. per acre); when potatoes, 436 kilog. per hectare (= 389 lbs. per acre); and when beet was grown for 3 years and maize for one year, the average annual loss of nitrogen by the soil over the four years, besides that removed in the crop, was 557 kilog. per hectare (= 498 lbs. per acre).

When ammonium sulphate was used, supplying annually 252 kilog. nitrogen per hectare (= 225 lbs. per acre), the annual losses were—after the green maize 359 kilog. per hectare (= 321 lbs. per acre), after the potatoes 555 kilog.

(= 496 lbs.), and after the beet and maize 615 kilog. per hectare (= 549 lbs. per acre) per annum.

Lastly, without any manure, the losses were, after 3 years of green maize 379 kilog. per hectare (= 338 lbs. per acre), after 3 years of potatoes 307 kilog. (= 274 lbs.), and after 3 years of beet and 1 year of maize, 476 kilog. per hectare (= 425 lbs. per acre) per annum.

Over the next 4 years, without further manure on the previously manured plots, and still without manure on the previously unmanured plots, the losses of nitrogen by the soil though still large, were generally much less. Besides that in the crops, they were per annum as follows:—

After farm-yard manure, with green maize 133 kilog. per hectare (= 119 lbs. per acre), and with potatoes 2 years and wheat 2 years 308 kilog. per hectare (= 275 lbs. per acre).

After nitrate of soda, with green maize 4 years 206 kilog. per hectare (= 184 lbs. per acre), and with potatoes and wheat, 38 kilog. per hectare (= 34 lbs. per acre).

After ammonium sulphate, with green maize, 148 kilog. per hectare (= 132 lbs. per acre), and after potatoes and wheat 140 kilog. per hectare (= 125 lbs. per acre).

Without manure for 7 years, the annual loss over the last 4 years was with maize 104 kilog. per hectare (= 93 lbs. per acre), whilst with potatoes and wheat there was a gain of 9 kilog. per hectare (= 8 lbs. per acre).

All the losses during the 3 years of the application of the manures, and especially those after 3 years of beet and one year of maize, are far in excess of anything that has come within our own knowledge and experience, and they are in amount such as reflection must show cannot possibly occur in actual practice.

For example, although it is estimated that the farm-yard manure supplied 1200 kilog. nitrogen per hectare (= 1071 lbs. per acre), in the 3 years, and that only 451 kilog. per hectare (= 403 lbs. per acre) were removed in the 3 crops of green maize, leaving a balance of the nitrogen of the manure of 749 kilog. per hectare (= 668 lbs. per acre), yet the surface soil was estimated to lose, not only this amount, but 116 kilog. per hectare (= 104 lbs. per acre) more, or in all 865 kilog. per hectare (= 772 lbs. per acre) in the 3 years. Again, with the same supply, 1200 kilog. nitrogen per hectare (= 1071 lbs. per acre) by manure, in 3 years, and the removal of 561 kilog. per hectare (= 501 lbs. per acre) in beet and maize in 4 years, leaving a balance from the manure of 639 kilog. (= 570 lbs.), the soil is estimated to have lost 2076 kilog. (= 1854 lbs.) more; or in all 2715 kilog. (= 2424 lbs.). It is true that when excessive amounts of farm-yard manure are applied there will probably be some loss by the evolution of free nitrogen, but here the estimated losses amounted to much more than the total nitrogen of the manure after deducting that removed in the crops. Indeed, M. DEHÉRAIN calls attention to the fact that, according to the figures, one-fourth of the total nitrogen of the surface soil has been lost! We repeat that such losses certainly do not occur in practical agriculture. But, if such loss could

take place with heavy manuring for 3 years, and the removal of 3 crops of beet and one of maize, what would be the result with ordinary manuring and cropping ?

How are these results to be explained? The accuracy of the analytical results recorded by M. DEHÉRAIN may be taken for granted. It seems to us, however, that in the method of taking the samples of soil for analysis, an explanation may be found; and we have the less hesitation in suggesting this, since we have found our own early results obtained under somewhat similar conditions, quite inapplicable for anything like accurate estimates of nitrogen per acre.

Perhaps it is no undue assumption to suppose that there has been more experience of soil sampling at Rothamsted than anywhere else; and we have, accordingly, learnt that very special precautions must be taken, when comparative estimates are to be made of the amount of nitrogen in the soil to a given depth, at different periods. When this is the object, it is absolutely essential that the samples taken should represent very exactly, both the same depth, and the same measure horizontally throughout the depth at the two periods. That is to say, it is essential that they should contain exactly the same proportions of the corresponding layers at the different dates; and this is the more important when the layer to be estimated includes both the manured and worked surface soil, and some of the unmanured and unworked subsoil.

Our own plan is to drive down a square iron frame, without top or bottom, having an exact measure superficially, and the exact depth for which the result is to be calculated. Even when this method is adopted, serious error may arise if at the different periods the soil is in a different state of consolidation, the result of manuring, the working of the land, the cropping, or the seasons. In other words, it is essential that a sample of a given area and depth should contain the same weight of dry soil at the two periods. We have given an illustration of the error possible, and of the correction necessary, when this is not the case, in a paper we published in 1882.*

Now, according to the description of his method given by M. DEHÉRAIN, he adopted the same plan as we did ourselves in our early experiments; that is, he took his samples, not by means of a frame of exact dimensions, but merely with a spade, with which it would be quite impossible to take a sample of exactly the same area throughout the depth adopted. Nor was the depth exactly the same in all cases. It is stated that it ranged from 25 to 30 cm. (= 9·8 — 11·8 inches), whilst the calculations per hectare are made for a depth of 35 cm. (= 13·8 inches). It is obvious, that if the samples were only taken to a depth of 25 or 30 cm., and upon the results obtained the calculations were made for a depth of 35 cm., the amount of nitrogen reckoned per acre, or per hectare, must be too high, as the subsoil from 25 or 30 cm. to 35 cm. deep would doubtless contain a much lower percentage of nitrogen than the layer above the depth of 25 or 30 cm. Indeed, M. DEHÉRAIN's determinations of nitrogen in the subsoils showed less than half as high a percentage as in the surface soils.

* "Determinations of Nitrogen in the Soils of some of the Experimental Fields, at Rothamsted, and the Bearing of the Results on the Question of the Sources of the Nitrogen of our Crops," pp. 32 *et seq.*

We trust, therefore, that M. DEHÉRAIN will accept our comments in all friendliness, when we say that our own dearly bought experience leads us to believe that the above facts are quite sufficient to render approximately accurate quantitative estimates at the different periods impossible. From the results, it seems probable that the samples taken at the commencement of the experiments in 1875 were less comparable with those of 1878 and 1879, than were those of these later dates with those of 1881 and 1885. The losses indicated were, indeed, in most cases, much less over the second period of 4 years; a result which is, however, doubtless partly due to the fact that no manure was applied during that period. Another reason for concluding that the samples were less truly representative at the commencement in 1875, than afterwards, is that the percentage of nitrogen found at that date (0·204), is high for the depth stated, of arable soil in ordinary agricultural condition. Though, if the soil is naturally very rich, or if it had been treated otherwise than in ordinary agricultural practice, such a percentage is by no means impossible. The percentages of 0·15 and upwards, as afterwards found, are however quite as high as is usual in good, but long worked arable soil, which is only manured and cropped in the ordinary way.

Then as to the amounts of nitrogen estimated to be gained by the soil to the depth of 35 cm. (=13·8 inches) by the growth of sainfoin for 5 years, and of mixed grasses for 2 years. They were, both on the plot where farm-yard manure had previously been applied, and on that which had been unmanured over the 7 years from the commencement, more than was taken off in the crops of that period. This is certainly more than our own experience would lead us to expect.

From his determinations of the nitrogen in the subsoils at different periods, M. DEHÉRAIN concluded that the gains were not from that source. The percentage in the subsoils of the different plots varied however considerably; and on this point it may be stated that in the subsoils at Rothamsted, the variations which are quite independent of manuring and cropping, are so great on the same plot, that we have found it quite impracticable to make calculations as to loss or gain in the total nitrogen of the subsoils.

Upon the whole we conclude, that certainly the estimated losses of the surface soils, and probably also the estimated gains, are higher than can possibly happen in practice; and that the results are due to the method of taking the samples of soil not being such as to ensure strictly comparable estimates at the different periods. At the same time there can be no doubt that there would be losses beyond those due to the removal of the crops, under the conditions in which losses were found; that is, when the land was under arable culture. Nor can there be any doubt that there would be gains in the surface soil when the land was laid down in sainfoin and mixed grasses; and M. DEHÉRAIN points out the practical significance of such facts.

M. DEHÉRAIN concludes that the loss of nitrogen by arable soil, that is by soil that is mechanically worked, is due to the slow combustion of the nitrogenous organic matter of the soil; the nitrogen being either evolved as free nitrogen, or oxidated

into nitric acid, and carried down into the subsoil, or into the drains. As to the gain by the surface soil, he considers that part is due to the action of deep-rooted plants, in taking up the nitric acid accumulated in the lower layers, and leaving a nitrogenous residue near the surface; a view in which we fully concur. As to gains not so to be accounted for, he considers it not yet settled whether they are due to the ammonia of the atmosphere, as supposed by M. SCHLŒSING, or to free nitrogen, as supposed by M. BERTHELOT.

In conclusion it may be remarked that, if the losses in ordinary agriculture were in amount anything like those which M. DEHÉRAIN'S figures show, even such large gains as are also indicated, would be far from sufficient to compensate them. It would indeed be necessary to seek for other sources of restoration, if our arable surface soils are not to lose their nitrogen much faster than we believe is the case in actual practice. That they do, however, slowly suffer reduction in their stock of nitrogen, when there is no restoration from without, there can we believe be no doubt. In other words, in actual practice, without such restoration from external sources, the losses are not fully compensated.

3. *The Experiments of* M. H. JOULIE.*

In this and subsequent sections, we have to consider evidence in regard to the fixation of free nitrogen, obtained, not in closed vessels, nor in the open field, but in vegetation experiments in which the soils and the plants were exposed to the free air, with known amounts of combined nitrogen supplied, and with more or less adequate precautions taken to exclude other sources than the free nitrogen of the atmosphere. M. JOULIE'S experiments are, in point of date, the earliest of those we have to notice.

M. JOULIE made two series of experiments on this subject. In the first he used a sandy-clay soil containing 0·104 per cent. of nitrogen, and in the second a sand containing only 0·0069 per cent. of nitrogen. In each series he used glass pots, with glass pans; in the first series he included 12 experiments, each in duplicate; and in the second series 10 experiments, also each in duplicate. Pounded glass was put at the bottom of each pot, and in each case 1500 grams of the matrix was used. Pure distilled water was supplied to the pans, which also received the drainage; and above the level of the water there were slits in the pots, for the aëration of the soil and the roots. The pots were placed on a bench under a glass roof, and were protected from birds and rain by means of wire gauze; the place of experiment being a court-yard of the Municipal Hospital, Rue Faubourg St. Denis, Paris. The different experiments represented so many different conditions as to manuring, those of Series 1, being as under:—

No. 1.—Without manure.

* 'Bulletin de la Société des Agriculteurs de France,' No. 1, Janvier 1, 1886, pp. 19-29.

No. 2.—A complete mineral manure, containing potassium sulphate and chloride, calcium sulphate, bicalcic-phosphate, and magnesium sulphate.

No. 3.—The complete mineral manure, and nitrate of soda = 0·3 gram nitrogen.

No. 4.—The complete mineral manure, and calcium carbonate.

No. 5.—The complete mineral manure, and caustic lime.

No. 6.—The mineral manure, excluding the bicalcic-phosphate, with nitrate of soda = 0·3 gram nitrogen.

No. 7.—The mineral manure, excluding the potash, with nitrate of soda = 0·3 gram nitrogen.

No. 8.—Farm-yard manure, containing 0·4 gram of nitrogen.

No. 9.—The same quantity of farm-yard manure, and calcium carbonate.

No. 10.—The complete mineral manure, and dried blood = 0·4 gram nitrogen.

No. 11.—The complete mineral manure, dried blood as in No. 10, and calcium carbonate.

No. 12.—Farm-yard manure as No. 8, and mineral constituents sufficient to bring the mineral supply up to that of the complete mineral manure.

On June 30, 1883, 6 germinated seeds of buckwheat were sown in each pot. There was great variation in the luxuriance of growth. On September 6, all the crops were cut at the level of the soil, leaving the roots in it. On September 15, after stirring the soils, each pot was re-sown with a mixture of rye-grass and hybrid trefoil. The rye-grass grew slowly through the winter, but the trefoil almost disappeared. In March, 1884, the herbage was cut, a little more rye-grass and trefoil was sown, and a second cutting was taken on June 18. On June 20 nitrate of soda = 0·1 gram nitrogen was supplied to Nos. 3, 6, and 7; and on August 21 the last cutting was taken.

The soils of the duplicate pots were mixed, as also were the crops, and the nitrogen was determined in the soils and in the crops separately. The following Table summarises the results obtained :—

SERIES 1.—Experiments with Sandy-clay Soil.

Experiments.	Manures.	Nitrogen.			Crops, dry.	Nitrogen.			Nitrogen, gain or loss.
		In soil.	In manure.	Total.		In soil.	In crops.	Total.	
		gr.	gr.	gr.	gr.	gr.	gr.	gr.	gr.
1	Without manure	1·56	..	1·56	11·00	1·685	0·3658	2·0508	+ 0·4908
2	Complete mineral manure	1·56	..	1·56	13·45	1·719	0·3540	2·0730	+ 0·5130
3	Mineral manure and nitrate	1·56	0·300	1·86	19·10	1·895	0·5163	2·4113	+ 0·5513
4	Mineral and calcium carbonate . . .	1·56	..	1·56	14·70	1·829	0·3390	2·1680	+ 0·6080
5	Mineral and caustic lime	1·56	..	1·56	13·80	2·042	0·3834	2·4254	+ 0·8654
6	Mineral without phosphate, and nitrate	1·56	0·300	1·86	14·42	1·599	0·4886	2·0876	+ 0·2276
7	Mineral without potash, and nitrate .	1·56	0·300	1·86	8·80	1·825	0·3110	2·1360	+ 0·2760
8	Farm-yard manure	1·56	0·400	1·96	14·35	1·752	0·3754	2·1274	+ 0·1674
9	Do., and calcium carbonate	1·56	0·400	1·96	14·85	1·857	0·3679	2·2249	+ 0·2649
10	Mineral, and dried blood	1·56	0·400	1·96	17·95	1·759	0·5564	2·3154	+ 0·3554
11	Mineral, dried blood, and calcium carbonate	1·56	0·400	1·96	12·80	1·523	0·4234	1·9464	− 0·0136
12	Farm-yard manure and mineral . . .	1·56	0·400	1·96	15·65	1·716	0·3814	2·0974	+ 0·1374

In the results given in the Table no account is taken of the nitrogen in the seed sown, which is estimated at not more than 2 to 3 millig. in the buckwheat, and the same in the rye-grass and trefoil, or in all not more than from 4 to 6 millig., which M. JOULIE thinks was largely compensated by leaves of the buckwheat carried away by the wind. He considers that the results are as exact as possible in experiments of the kind. He concludes that, as in M. BERTHELOT'S experiments, the results establish the reality of the fixation of free nitrogen in the presence of clay; and further, that the fixation takes place to a greater extent in the presence of vegetation, when the conditions are favourable for the development of the plants.

In the second series of experiments sand instead of soil was used. The percentage of nitrogen in it was only 0·0069, so that the actual amount of combined nitrogen supplied in the 1500 grams put into each pot, was only 0·1035 gram.

This series included 10 conditions as to manuring, each in duplicate. Each pot of experiments 1 to 8 received the complete mineral manure—No. 1 alone; Nos. 2, 3, 4, and 5, each with 0·3 gram of nitrogen as nitrate of soda; No. 6 with 0·2 gram nitrogen as nitrate of soda (half applied at the commencement and half a month later); No. 7 with 0·2 gram nitrogen as ammonium sulphate; No. 8 with 0·3 gram nitrogen as dried blood; No. 9 received farm-yard manure = 0·3 gram nitrogen, with mineral constituents sufficient to bring the mineral supply up to that by the complete mineral manure; lastly, No. 10 received at the commencement 0·3 gram nitrogen as powdered hay, and later 0·1 gram as nitrate of soda; the mineral composition of the manure being made up as in the case of the farm-yard manure.

On May 25, 1884, 10 germinated seeds of buckwheat were sown in each of the pots, excepting those of Experiment 2, which received only 5, and those of Experiment 3 which received 15. On September 16 the plants were cut, and they and the soils were analysed, the duplicates being mixed as before. The results are given in the following Table.

SERIES II.—Experiments with Sand as Soil.

Experiments.	Manures.	Nitrogen.			Crops, dry.	Nitrogen.			Nitrogen, gain or loss.
		In Soil.	In Manure.	Total.		In Soil.	In Crops.	Total.	
		gr.	gr.	gr.	gr.	gr.	gr.	gr.	gr.
1	Complete mineral manure	0·1035	. .	0·1035	0·970	0·1455	0·0290	0·1745	+ 0·0710
2	Do., and nitrate soda	0·1035	0·300	0·4035	6·825	0·3270	0·1405	0·4675	+ 0·0640
3	Do., do.	0·1035	0·300	0·4035	6·585	0·3060	0·1680	0·4740	+ 0·0705
4	Do., do.	0·1035	0·300	0·4035	5·890	0·4080	0·1375	0·5455	+ 0·1420
5	Do., do.	0·1035	0·300	0·4035	7·850	0·2280	0·2525	0·4805	+ 0·0770
6	Do., do.	0·1035	0·200	0·3035	7·612	0·2280	0·2420	0·4700	+ 0·1665
7	Do., and ammonium sulphate . .	0·1035	0·200	0·3035	6·425	0·1850	0·2315	0·4165	+ 0·1130
8	Do., and dried blood	0·1035	0·300	0·4035	5·600	0·2685	0·1375	0·4060	+ 0·0025
9	Do., and farm-yard manure . . .	0·1035	0·300	0·4035	4·572	0·4350	0·1205	0·5555	+ 0·1520
10	Do., powdered hay, and nitrate soda	0·1035	0·400	0·5035	1·225	0·3570	0·0390	0·3960	− 0·1075

In the experiments of the first series, with a range of from 1·56 to 1·96 gram of combined nitrogen supplied, there were, in several cases, indicated gains of 0·5 gram, or more; and in one case the gain amounted to 0·865 gram. In the experiments of the second series, with a total supply in sand and manure generally ranging from 0·3 to 0·5 gram, there was, in one case a loss, in five cases the gain was less than 0·1 gram, and in no instance did it reach 0·2 gram. M. JOULIE attributes the less amount of gain in the second series, to the much shorter period of vegetation involved.

Reviewing the results of the two series of experiments, M. JOULIE says the variable quantities of nitrogen gained cannot be attributed to dust, ammonia, or other compounds of nitrogen, in the air, as all the pots were equally exposed to these; whilst there is a range from 0·1075 gram loss, to 0·8654 gram gain of nitrogen, the difference amounting to nearly 1 gram. The result must be due, therefore, to the fixation of the free nitrogen of the air, either in the soil or by the plant. M. BERTHELOT attributed the result in his experiments to the clay soil, under the influence of microbes; but M. JOULIE cannot go so far. It was, however, true that, in his experiments, the surface of the water in the pots and the surface of the soils showed myriads of microbes. He asks—if such bodies can cause the fixation of free nitrogen, why should not grouped cells, as in the case of the higher plants, have the same power?

He further says—as plants have the power of causing the combination of carbon with the elements of water, after having decomposed carbonic acid, whilst chemists can only reduce it to carbonic oxide; as MM. THENARD have succeeded in bringing nitrogen into combination with the elements of water; and as M. BERTHELOT has shown that free nitrogen is brought into combination with dextrine and cellulose under the influence of the silent electric discharge—it is only a logical consequence that free nitrogen should be brought into combination within the plant. In reference to this argument it may be oberved that the parallelism of the action by which free nitrogen combines with the elements of water in the laboratory, with that by which carbon and the elements of water combine within the plant, only holds good on the assumption that the carbon of the carbonic acid is first reduced to the free state, and so combines with the elements of water, without the intervention of its own oxygen.

M. JOULIE compares the amounts of nitrogen fixed in his various experiments with the amounts of crop produced, and observes that the gains have no relation to the amount of vegetation. He next comments on the connection between the condition of manuring of the various soils, and the amounts of nitrogen gained. Referring to the results of the first series of experiments, he points out that whilst without manure the gain was 0·491 gram, it was raised to 0·513 gram by purely mineral manure. Again, whilst the addition to the mineral manure of 0·3 gram nitrogen as nitrate of soda only gave a further gain of 0·038 gram, and the addition of calcium carbonate increased the fixation by only 0·0950 gram, the addition of caustic lime increased the fixation by 0·352 gram. It appeared, therefore, that lime exercised a very favourable influence on the phenomenon.

On the other hand, the use of organic matter, as farm-yard manure, or dried blood, much reduced the amount of fixation. The addition of calcium carbonate to the farm-yard manure increased the fixation, whilst the same addition to the dried blood reduced it.

When in the first series of experiments either phosphoric acid or potash was excluded from the mineral manure, there was a most remarkable decline in the amount of fixation, indicating, M. JOULIE thinks, how necessary is a due balance of the mineral supplies for the full development of the action.

In the second series, as in the first, the unfavourable influence of organic manures was obvious.

M. JOULIE concludes that his own results, like those of M. BERTHELOT, show that the fixation of nitrogen is due to a physiological action. Microbes play an important part; and his own experiments show that the action is developed in the absence of clay. His results were not very favourable to the supposition that the plants themselves effected the fixation; but he considers that further comparative experiments, with and without vegetation, are necessary to settle the point. For the present he limits himself to the establishment of the great fact of the fixation of the free nitrogen of the atmosphere, leaving to the future the exact explanation.

In order to show the practical importance of the fixation of free nitrogen, M. JOULIE takes for illustration the results of the experiment No. 5, in the first series, in which the largest amount of gain was indicated. In that experiment the complete mineral manure, with caustic lime in addition, was used, without any artificial supply of nitrogen. At the commencement the soil contained 1·56 gram, and at the conclusion 2·042 grams of nitrogen, and the crops contained 0·3834 gram, showing a total gain, therefore, of 0·8654 gram nitrogen. As the soil in the pots was 10 cm. deep, he calculates that this would correspond to 1144 kilograms of nitrogen fixed per hectare weighing 2000 tonnes (= 1021 lbs. nitrogen per acre). Or, reckoning only according to the relative superficies of the soil in the pots, and of a hectare, the gain of nitrogen would be 432 kilograms per hectare (= 386 lbs. per acre). He further reckons, that the value of the nitrogen gained, at 1 franc 50 per kilogram as in manures would be 1716 francs, or 650 francs per hectare (= 555 or 210 shillings per acre), according to the mode of calculation adopted. He admits, however, that it cannot be estimated so high, because the nitrogen fixed in the soil is in a form not at once assimilable by plants.

In reference to the above results, M. JOULIE says that our own at Rothamsted, and those of M. DEHÉRAIN in France, obtained in field experiments, cannot be relied upon as the basis of conclusions on this subject; because the samples of soil taken at different times cannot exactly represent the mean composition of the soil, and because the layer of soil sampled may have lost combined nitrogen by drainage, or gained it from the subsoil. M. JOULIE, on a former occasion, indicated in more detail his

objection to our mode of experimenting; but he did so in a way which showed entire ignorance or misconception of our method.

We have already given our reasons for believing that certainly the losses, and probably the gains also, shown in M. DEHÉRAIN's experiments were too high. We nevertheless, quite agree that there would be losses where he found losses, and that there would be gains where he found gains. It is to be observed, however, that it was under the conditions of arable culture, that is of artificially aërated soil, and with vegetation, that M. DEHÉRAIN found great losses, whilst it is in well aërated soils, also with vegetation, that M. JOULIE finds such enormous gains.

It is further to be observed that the large gains shown in M. JOULIE's results were obtained chiefly in the growth of buckwheat, and not with plants of the Leguminous family which are reputed to be "*Nitrogen collectors.*" From our own results, taken together with known facts as to agricultural production, and the fertility of soils, it may be confidently affirmed that such gains as M. JOULIE finds within a period of about 14 months, do not take place, either with or without vegetation, in ordinary soils, in ordinary practice.

4. *The Experiments of* Dr. B. E. DIETZELL.

At the meeting of the *Naturforscher-Versammlung* at Magdeburg in 1884, Dr. DIETZELL gave the results of experiments, the primary object of which was to determine whether plants absorb combined nitrogen from the atmosphere by their leaves; but they equally afford evidence on the question whether they assimilate the free nitrogen of the air. The plants selected were peas and clover, each of which he grew under four conditions as to manuring. A garden soil, containing 0·415 per cent. of nitrogen was used, and the experimental pots were made of hard burnt clay. The plants were watered with distilled water, and the drainage was returned to the soils. The pots and their contents were exposed to free air, but protected by a linen roof.

The conditions of the different experiments were as follows:—No. 1, without manure; No. 2, manured with kainite; No. 3, with kainite and superphosphate; No. 4, with kainite, superphosphate, and calcium carbonate; No. 5, with kainite, superphosphate, and calcium carbonate, but without a plant; and No. 6, without either manure or plant.

The nitrogen was determined in the original soil, and in the seed; also in the soil at the conclusion of the experiment, and in the plants grown. The following figures show the losses or gains of nitrogen, represented in percentage of the original nitrogen in soil and seed:—

	Loss or gain of nitrogen.		
	Peas.	Clover.	Without plant.
	per cent.	per cent.	per cent.
Without manure	− 10·69	− 5·10	+ 0·26
Kainite	− 15·32	− 14·76	
Kainite and superphosphate	0·00	− 7·37	
Kainite, superphosphate, and calcium carbonate	− 12·72	− 10·38	− 10·24

Thus, there was, in every case but one with the peas, and in every case with the clover, a loss, not a gain, of nitrogen. There was also a loss where the soil was manured, but left without a plant.

On the other hand, in the experiment without either manure or plant, the figures show a gain of nitrogen. In reference to this last result, it should, however, be stated, that whilst in one German account it is, as in the Table, given as a gain of only 0·26 per cent. of the original nitrogen, in another German account, as well as in an English one, it is represented as a gain of 0·26 gram. In the first case the gain would be immaterial, whilst in the other it would be considerable, though still but small compared with the results obtained by M. JOULIE.

It is to be observed that whilst with almost exclusively non-leguminous growth, M. JOULIE found gains of nitrogen in all cases, and in some very large gains, Dr. DIETZELL, experimenting exclusively with leguminous plants, which are credited with being beyond all others atmospheric nitrogen accumulators, in all cases found losses instead of gains. How is this discrepancy to be explained? It may be answered, that with a garden soil containing so much as 0·415 per cent. of nitrogen, it is not at all surprising that there should be some loss. Indeed loss would seem to be a perfectly natural result; and it is obvious that, neither from the combined nitrogen of the atmosphere, or that due to accidental sources, nor from free nitrogen, either directly or indirectly, did these reputed nitrogen-collectors gain nitrogen enough to compensate the losses from the rich soil. It is, indeed, recorded gains that require confirmation, with very careful methods of experimenting, before they can be accepted as conclusive evidence of the fixation of free nitrogen, and not as due merely to accidental sources of combined nitrogen, or to other experimental errors almost inevitable in experiments in which the soils and the plants are not enclosed, but exposed to the free air.

In conclusion, all the results of Dr. DIETZELL, excepting the one in which he found a gain, seem quite accordant with well established facts. On the other hand, if free nitrogen is really fixed in the soil under the influence of microbes, it certainly might be supposed that the result would be developed in a soil so rich in organic matter, and doubtless, therefore, in micro-organisms also, as a garden soil containing 0·415 per cent. of nitrogen; and especially might it be supposed that it would be developed in

the presence of leguminous growth, in connection with which, if at all, the establishment of the reality of such an action would serve to explain facts as yet not otherwise fully explained.

5. *The Experiments of Professor* B. FRANK.

In the number of the 'Berichte der Deutschen Botanischen Gesellschaft' for August, 1886, Dr. FRANK gave a paper, "Ueber die Quellen der Stickstoffnahrung der Pflanzen." At the meeting of the *Naturforscher-Versammlung*, held at Berlin, in September, 1886, he gave a further communication on the subject; and he has since published a paper on the position of the question, before, at, and after that meeting.

He admits the probability of the conclusion of BOUSSINGAULT and others, that plants do not directly assimilate free nitrogen. He states, however, that in practical agriculture it is assumed that some plants do fix the free nitrogen of the air; and he refers to the experience and writings of SCHULTZ-LUPITZ, and others, on the point, especially during the last 10 years. Thus, SCHULTZ-LUPITZ found that certain Leguminosæ, especially lupins, grew well in a poor soil, under the influence of mineral manures; and so far from appearing to exhaust the soil, cereals, roots, and potatoes, grew well after them, as they would if nitrogenous manures had been applied.

FRANK refers to the amount of combined nitrogen coming down in rain, &c., as about 3 kilograms per hectare (= 2·7 lbs. per acre), per annum, and to the average amount of nitrogen removed in crops as 51 kilograms per hectare (= 45·5 lbs. per acre), apparently obtained from the air by the nitrogen-gathering plants, which are considered more effective than manure and cattle feeding. He points out that the evidence is not conclusive, and he recognises that the question is, whether this is only "*Raub-bau*" after all? This can only be settled by direct experiments.

He had been working at the subject for three years, and now gives the results of the last completed experiments, those of 1885. The first question to be decided was—do the so-called nitrogen-gathering plants enrich the soil, whilst the same soil, with the same exposure, but without a plant, does not gain combined nitrogen?

He experimented with a humus-sand soil, finely sifted. In some cases he used cylinders of pottery glazed inside, 80 cm. (= 31·5 inches) deep, and 17·5 cm. (= 6·9 inches) wide; in others glass cylinders, also of 80 cm. deep, but only 11 cm. (= 4·3 inches) wide. To the rim of each cylinder a cap of wire gauze was fixed, to exclude insects; and the vessels were exposed to free air. The soils were watered with distilled water. One of the wide earthen cylinders, and two of the narrow glass cylinders, were left without a plant. In one wide earthen cylinder three lupin seeds were sown; in one narrow glass cylinder two lupin seeds, in another one lupin seed only, and in another one lupin seed and 20 incarnate clover seeds were sown. If weeds grew where there was no experimental plant, they were stocked up, but if in the vessels with the experimental plants, they were undisturbed.

The nitrogen was determined in seed, in the products of growth, and in the soils before and after growth. The seeds and plants were dried at 50° C. for analysis, and it was found that the lupin seeds contained an average of 0·009 gram. nitrogen per seed, and that 20 incarnate clover seeds contained 0·003 gram of nitrogen.

Summary of Dr. FRANK's results in the summer of 1885.

	Nitrogen.						
	Per cent. in soils.		Total in soil and seed at commencement.	In the plants.	Total in soil and plants at conclusion.	Gain or loss on the original quantity.	
	Before experiment.	After experiment.				Actual.	Per cent.
	Per cent.	Per cent.	Grams.	Grams.	Grams.	Grams.	Per cent.
1. Earthen cylinder without plant	0·0957	0·0907	20·5755	—	19·5112	−1·0643	− 5·1
2. Glass cylinder without plant .	0·0957	0·0837	9·2589	—	8·0979	−1·1610	−12·5
3. Glass cylinder without plant .	0·0957	0·0832	7·1775	—	6·2400	−0·9375	− 8·69
4. Earthen cylinder with 3 lupin seeds	0·0957	0·1065	20·5547	0·8208	23·6758	+3·1211	+15·2
5. Glass cylinder with 1 lupin seed	0·0957	0·0992	9·0373	0·1138	9·4781	+0·4408	+ 4·87
6. Glass cylinder with 1 lupin, and 20 incarnate clover seeds	0·0957	0·0854	8·29039	0·2295	7·6169	−0·67349	− 8·08
7. Glass cylinder with 2 lupin seeds	0·0957	0·0893	8·8032	0·0274	8·2251	−0·5781	− 6·56

It is seen that the actual amounts of nitrogen involved were large, being about 20 grams in the experiments in the wider vessels, and nearly half as much in those in the narrower vessels. The losses were in some cases about, or more than, a gram; and one of the two gains amounted to more than 3 grams.

In each of the three experiments without a plant (Nos. 1, 2, and 3), there was a loss of nitrogen. FRANK states that it was not as nitric acid, which either diminished but little, or increased, there being no plant to take it up. Nor was it as ammonia, as a direct experiment with a rich peaty soil, enclosed under a luted bell jar, and ammonia free air passed through it, showed very little ammonia evolved. He points out that the more imperfect the ventilation of the soil, the less was the gain; and he considers it probable that in the absence of ventilation the evolution of free nitrogen would be enhanced. In fact the losses were greater in experiments 2 and 3 with vessels 80 cm. (= 31·5 in.) deep, and only 11 cm. (= 4·3 in.) wide, than in No. 1 with a width of 17·5 cm. (= 6·9 in.).

It is indeed obvious that, with vessels so narrow and deep, and closed at the bottom, as according to the description we conclude they were,* and with no plant to cause evaporation, and with consequently very little aëration, the conditions would be favourable for the evolution of free nitrogen, and the more so in the narrower vessels. In fact it may be doubted whether, if there had been holes at the bottom of the

* We have since ascertained that the vessels were closed at the bottom.

vessels, and free aëration had been kept up, there would have been any loss at all from a soil containing, as this soil did, less than 0·1 per cent. of nitrogen.

It is further to be observed that the losses with growth were, in No. 6, where the one lupin plant died before blooming, and where only 7 of the 20 incarnate clover seeds grew, and in No. 7 where only one of the two lupin seeds grew, and it gave very small produce. In both cases, therefore, there would, independently of the soil itself, be decomposing organic matter, conditions under which, in the experiments of BOUSSINGAULT, and also in those at Rothamsted, there was more or less loss, supposed to be as free nitrogen.

Again, in the experiment above referred to, made to determine whether there was any material loss as ammonia, FRANK used a very unusually rich soil, containing 1·1836 per cent. of nitrogen, which after exposure for 180 days to a current of air in shallow vessels, only contained 1·0976 per cent. It had lost therefore 0·0860 per cent., corresponding to 7·27 per cent. of its total nitrogen, of which only 0·0004 was as ammonia. In reference to this loss FRANK says that if such loss is always going on in the soil, we must suppose that there is restoration in some way. But it is to be observed that the soil in which he found such loss was not only about 12 times as rich as the one used in his other experiments, but probably 8 or 10 times as rich as the majority of ordinary arable soils. Hence it is obvious that the amount of loss it sustained, cannot be taken as any indication of what happens in actual practice. Nor can the conditions of the experiments in the narrow and deep vessels without ventilation, be considered comparable with those of ordinary arable surface soils, or even of subsoils with fairly good natural, or with artificial drainage.

That soils do lose nitrogen, not only by the removal of crops, but also by drainage of nitric acid, is certain; and if there is no return of nitrogenous manures from without, the result is a gradual diminution of the fertility, so far as the nitrogen is concerned. But the balance of evidence is against the supposition that there is a constant and considerable loss by the evolution of free nitrogen, in the case of arable soils which are only moderately rich in nitrogenous organic matter, and which are fairly drained, either naturally or artificially.

On this point it may be mentioned that, in those of the field experiments at Rothamsted in which the unusual practice of applying farm-yard manure every year is adopted, it is found that there is considerable loss of nitrogen from the soil, beyond that known to be removed in the crops, and estimated to be lost in the drainage. On the other hand, where no nitrogen has been applied for many years, and the amount of nitrogen in the surface soil is only about, or little more than, 0·1 per cent., the loss of nitrogen by the soil over a long series of years corresponded approximately with the amounts removed in the crops, together with those estimated to be lost in the drainage. Again, when ammonium-salts are applied, even so late in the season as October or November, and drainage takes place soon afterwards, the drainage-waters will contain amounts of nitrogen showing a very direct relation to the different

amounts of ammonia applied in the manure; but scarcely any of it as ammonia, nearly the whole existing as nitric acid; and this is the case although the drainage passes through 20 inches or more of raw clay subsoil. Lastly, direct experiments have shown that there is a diminution in the amount of nitric acid in the soil down to a certain depth, varying according to the root-range of the crop grown, and to the season, but that in the depths of the subsoil below this point, the amount is again greater.

Upon the whole, then, we are disposed to think that, in most arable soils which are only manured and cropped as in ordinary practice, and which have fair natural or artificial drainage, there is little if any loss by the evolution of free nitrogen.

We would indeed submit, that the losses found by Dr. FRANK in his series of 7 experiments, are in all probability largely, if not entirely, accounted for by the special conditions of the experiments themselves to which attention has been called, and that those found in the rich soil, in the closed vessel, depended greatly if not wholly on the abnormal character of the soil itself.

The gains (as in Experiments 4 and 5) are, however, by no means so easy to explain. Indeed, if there were no accidental source of error, such as all vegetation experiments in free air must be more or less liable to, the explanation obviously would be, that the free nitrogen of the air had come into play in some way.

Dr. FRANK supposes that, even in Experiments 6 and 7, where a loss was indicated, there had nevertheless been a gain under the influence of the plant growth, but not sufficient to counterbalance the loss. We would suggest that, in Experiments 4 and 5, where a gain was indicated, there may have been no loss at all; especially in No. 4, with the wider vessel, and where the growth of the lupins was the most luxuriant, and the seed ripened; for, under such conditions, there would be much more evaporation, and therefore much more movement within the soil, and aëration of it.

But, apparently giving full force to the evidence in his experiments of loss by the evolution of free nitrogen, and taking it as confirmation of the supposition that in actual practice soils suffer to a very material extent in this way, Dr. FRANK says that all that can be concluded with certainty is—that two opposite actions are at work in the soil—one setting free nitrogen, and the other bringing it into combination—the latter being favoured by the presence of vegetation. He admits that neither his own results, nor those of others, afford decisive evidence as to how this takes place; nor does he think that it follows from his results, that the plant itself effects the combination.

Independently of direct experimental evidence on the point, he considers it unlikely that the gain of nitrogen can be due to the ammonia of the air, because it is so small in amount, because the gain is by the soil rather than by the plant, and lastly, because, as the ammonia of the air is largely due to emanations, if it were the source we should be without explanation of the circulation of nitrogen in nature; that is, of the return of free nitrogen into combination, to compensate for the losses by its evolution from combination.

Indeed, with Dr. FRANK, as with other investigators of this subject, a prevailing idea seems to be, that there must exist a source of compensation for the loss of combined nitrogen by the removal of crops, by drainage, and above all by the evolution of free nitrogen from the soil, and in other ways. We believe, however, that the losses by the removal of crops are much exaggerated, due account not being taken of the return by the manures of the farm; also that the loss by the evolution of free nitrogen by the soil is exaggerated, the results obtained in the laboratory not being comparable with the conditions in the field. At the same time we believe, that such losses as do in reality take place in ordinary agriculture, are not fully compensated; but that arable soils yielding products for sale, and not receiving nitrogenous manures from without, do gradually reduce in fertility, so far as their nitrogen is concerned.

6. *The Experiments of Professor* HELLRIEGEL *and Dr.* WILFARTH.

At the Berlin meeting of the *Naturforscher-Versammlung*, held in September, 1886, in the Section *Landwirthschaftliches Versuchswesen*, Professor H. HELLRIEGEL gave a paper entitled, "Welche Stickstoffquellen stehen der Pflanze zu Gebote?" One of ourselves was presiding at the time, and the communication was obviously considered by the numerous agricultural chemists present to be one of great interest and importance. We have vainly tried to get the paper *in extenso*;* but we have now two accounts of the results, one by HELLRIEGEL himself, in the 'Zeitschrift des Vereins für die Rübenzucker-Industrie des Deutschen Reiches,' and another in a very comprehensive summary of the evidence relating to the sources of the nitrogen of vegetation, published by Professor KÖNIG.†

HELLRIEGEL first gave results of experiments with barley, oats, and peas, made in pure washed sand, in pots 20 cm. deep, each containing 4 kilograms of the material. Nutritive solutions containing no nitrogen were added to all. One series of pots received besides, a fixed quantity of nitrogen as nitrate of soda, a second twice as much, and a third four times as much. The results showed that in the case of the gramineous plants the amount of produce grown had a direct relation to the quantity of nitric nitrogen supplied. It was very different with the peas.

In many comparative experiments he got astonishing growth with peas in the sand with all other food substances, but without nitrogen, whilst under exactly similar conditions the Gramineæ showed nitrogen-hunger and failed. He gives the following results with peas:—

* In an interview with Professor HELLRIEGEL, at the meeting of the *Naturforscher-Versammlung*, held at Cologne in September, 1888, we learnt that the details of his experiments have not yet been published, but that a full paper is in course of preparation. [*October*, 1888.]

† 'Wie kann der Landwirt den Stickstoff-Vorrat in seiner Wirtschaft erhalten und vermehren?' Berlin, 1887.

	Total above-ground dry substance.	Seed produced.
	grams.	grams.
1884	28·483	13·947
1885	27·816	11·710
1885	33·147	12·426
1886	20·372	8·956

To convey an idea of what 33 grams of dry produce means, he states that with barley that amount was never obtained even with the addition of nitrates. So far as we know, he has not estimated the amount of nitrogen in the produce,* which, however, would be large; and as to the source of it, he says it is obviously from the air.

The quartz sand was washed many times, the nutritive mixture given contained no compound of nitrogen, and the plants were watered with distilled water (the first third of the distillate not being used). Hence he considered the supposition that accidental impurity was the source of the nitrogen to be out of the question, especially when the amount of the produce is considered. Further, the constant failure of the Gramineæ under exactly the same conditions, afforded direct proof that the soil was not the source of the nitrogen. He concludes:—

The Papilionaceæ are distinguished from the Gramineæ in not being dependent on the soil for their nitrogenous food. The sources of nitrogen which the atmosphere affords have, for these plants, the highest importance. They alone can suffice to bring them to a normal or full development.

To determine how far the combined nitrogen in the atmosphere was the source, he made a series of 4 experiments, in each of which a pot of peas was enclosed under a glass shade, and a constant stream of air was passed through; in No. 1, without first washing the air, but in Nos. 2, 3, and 4, the air was washed to remove all nitric acid and ammonia. The result was, that the growth was as good with the washed as with the unwashed air. There only remained, therefore, the supposition that the Papilionaceæ have the power of utilising free nitrogen. He accepts the conclusion of BOUSSINGAULT that the Papilionaceæ cannot directly assimilate free nitrogen; but the possibility of an indirect action is not thus excluded. In the first place BERTHELOT had shown that bacteria abound in the soil, and possess the power of bringing free nitrogen into organic combination; and secondly the nodules found on the roots of normally growing Papilionaceæ are full of bacteria.

The author has often observed that when peas are grown in a nitrogen-free soil, the growth is quite normal, and the colour of the leaves quite healthy, until the reserve material of the seed is used up. Growth is then arrested, and the leaves become pale or yellow; but after a shorter or longer time, they regain their green colour, a second period of growth begins, and it continues to the end. In a series of parallel experiments, however, some plants will develop full, and others only a very limited growth.

* See foot-note, p. 64.

Examination showed that the plants which did not develop beyond the first period, had either no nodules on their roots, or only weak indications of them; whilst the roots of the plants which developed favourably, had the nodules, and the more, or the older and stronger, the nodules, the better was the development of the plants. He, therefore, instituted experiments to determine whether by the supply of the organisms the formation of the root nodules and favourable growth could be induced; and on the other hand, whether by their exclusion the result could be prevented.

To each of ten out of 40 experimental pots, with nitrogen-free soil, 25 c.c. of an extract of fertile soil, made with five times its weight of distilled water, was added. After a time the plants in each of the ten pots regained their green colour, and grew vigorously; whilst in only two of the thirty pots without the addition of the micro-organisms did the plants develop favourably, all the rest showing more or less nitrogen-hunger, and some were quite yellow. After a time the plants from two of the pots with bacteria, and from five without, were taken up, and examination showed very strikingly the connection between the amount of above-ground growth, and the development of the root nodules. In the 8 remaining pots with bacteria, the growth was very uniform; whilst in only 4 of the remaining 25 without bacteria was there fair development.

HELLRIEGEL states that the quantity of soil extract added contained only 1 millig. nitrogen. In two other experiments everything was sterilised. The peas germinated healthily, developed their first 6 leaves, but did not go further, and died, not a trace of nodules being found on their roots. He concludes:—

To the nourishment of the Papilionaceæ, especially their assimilation of nitrogen, the so-called leguminous nodules, and the micro-organisms they contain, stand in close and active relation.

It was remarkable, however, that in numerous trials with lupins, under exactly similar conditions, successful second growth could not be obtained. The conclusion was, that the organisms in the root nodules of lupins were of another kind, which were less generally distributed than those found in the nodules of peas. Further, it is known that lupins do not grow well on a heavy, or even on a rich humus soil.

Three rows of the experimental pots were filled with quartz sand, a nitrogen-free solution was added, and lupins were sown. A portion of diluvial sandy soil, where lupins were growing well, was treated with 5 times its weight of distilled water, and a turbid extract was obtained, in which it might be assumed that there would be a sufficient quantity of the micro-organisms peculiar to the sandy soil. To each experimental pot of the first row, 25 c.c. of this fluid was added; those of the second row were left without any such addition; and those of the third row received the extract from the loamy humus soil, the same as was used in the case of the experiments with the peas.

The germination and early growth were favourable in all cases; then followed the hunger-condition, and after 30 days from the sowing, all the rows showed equal poverty. Then the lupins of the first row began to show fresh green colour, assumed

a healthy aspect, and grew well. The plants of both the second and third rows maintained a sickly, brown-red colour.

The roots of the plants of the first row, which received the sandy-soil extract, were thick with large root-nodules, such as are found in the field with normal vegetation. On the roots of the second series, without any soil extract, no trace of the nodules could be found; and on the roots of the third series, which received the extract from the rich soil, on only one plant was a single weak nodule observed.

Other sandy-soil Papilionaceæ, such as serradella (*Ornithopus sativa*), behaved as the lupins; whilst peas, vetches, and beans, grew best in the third row, and red clover gave no special result.

HELLRIEGEL observes that, although the Papilionaceæ have the property of turning to account atmospheric nitrogen, they nevertheless do take up nitrogen from the soil, especially as nitrates; but he considers it doubtful whether such plants can attain to a normal development with nitrates alone, and with the exclusion of micro-organisms.

Finally, he admits that his observations require control and perfecting in various directions; and he confines himself for the present, to the simple statement of his experimental results.

In reference to the foregoing results of HELLRIEGEL, we have already said that we have not been able to find any record of the experimental details; and indeed it seems doubtful whether determinations of nitrogen were made, either in soils, seed, or products of growth.* Nevertheless, such particulars as are given, can leave no doubt whatever that the products of growth, both of the peas and of the lupins, where favourable conditions were provided, would contain very much more nitrogen than the seed sown. If, therefore, the washed sand, and the nutritive solutions, were free from combined nitrogen, and the conditions of exposure of the experimental pots in free air were such as to exclude the possibility of the access of accidental sources of combined nitrogen, the obvious explanation is that the nitrogen gained had its source in the free nitrogen of the air.

Then, as to the conditions under which the free nitrogen has been brought into combination? The negative result with the Gramineæ, the negative result with the peas when everything was sterilised, or when the sand was not seeded by the soil-extract, the positive result with the peas when the sand was seeded by the humus soil extract, the negative result with the lupins when their soils were not seeded, or when they were seeded with the same extract as the peas, and the positive result when seeded with the extract from the sandy soil where lupins were growing, seem to exclude any other conclusion than that the micro-organisms supplied by the soil extracts were essential agents in the process of fixation. Further, the development of nodules on the roots was, to say the least, a coincident of the fixation. To HELLRIEGEL's conclusions on this point, the objections have been raised,—first that the nodules are a result and not a cause of active growth, and that in fact they con-

* See foot-note, p. 64.

stitute a supply of reserve material for growth; and secondly that the investigations of TSCHIRCH and BRUNCHORST, prove that the nodules have no external communication with the soil. As to the latter objection, it may be observed, that Professor MARSHALL WARD has recently shown that, on the death of the nodules, the micro-organisms become distributed within the soil; and further, that in the case of HELLRIEGEL'S experiments it was the organisms themselves, or their germs, that he supplied to the soil.

It must be admitted that HELLRIEGEL'S results, taken together with those of BERTHELOT and others, do suggest the possibility that although the higher plants may not possess the power of directly fixing the free nitrogen of the air, lower organisms, which abound within the soil, may have that power, and may thus bring free nitrogen into a state of combination within the soil in which it is available to the higher plants —at any rate to members of the Papilionaceous family. At the same time it will be granted, that further confirmation is essential, before such a conclusion can be accepted as fully established.

Since the above was written, Dr. WILFARTH, who was associated with Professor HELLRIEGEL in the experiments which have been described, has given an account of a subsequent series of experiments which were made in 1887. Under the title of "Ueber Stickstoffaufnahme der Pflanzen," he gave the results at the meeting of the *Naturforscher-Versammlung*, at Wiesbaden, in September, 1887, and a short account of them was published in the 'Tageblatt,' pp. 362–63; and also in 'Versuchs-Stationen,' vol. 34, 1887, pp. 460–61, of which the following is a pretty full summary:—

He states that the previous experiments had shown, that the Gramineæ, the Chenopodiaceæ, the Polygoneæ, and the Cruciferæ, take their nitrogen from the soil, and that their growth was proportional to the available supply of nitrogen. The Papilionaceæ on the other hand take their nitrogen from the air, and grow quite normally in an absolutely nitrogen-free soil, provided a very small quantity of cultivated soil be added. He further states that they have now repeated the experiments in the same way, and that the results fully confirm the conclusions before arrived at as above stated.

The new experiments were made with oats, buckwheat, rape, peas, serradella, and lupins. The experimental soil was a pure sand, entirely free from nitrogen. Each pot contained 4 kilog. of this sand, to which were added the necessary mineral constituents. All the plants grew until the nitrogen of the seed was used up. Then to each pot a small quantity of the turbid watery extract of a surface soil was added, the quantity representing 5 c.c. of the soil, and containing from 0·3 to 0·7 millig. nitrogen. After this the different plants exhibited very great differences in growth. Neither the oats, rape, nor buckwheat showed any effect from the addition of the soil-extract, but remained in the condition of "nitrogen-hunger." On the other hand, the Papilionaceæ after a time recovered from their nitrogen-hunger, suddenly became dark green, and then grew luxuriantly up to ripeness. In experiments in which the soil-extract was sterilised by boiling, there was no such result. Peas grew well under

the influence of extract from any cultivated soil, but lupins and serradella only when extract from a soil where these plants were growing was used. The series comprised 178 pots, and the results were so accordant, as was shown by photographs exhibited, that the possibility of accident, or nitrogenous impurities, was out of the question.

Thus, it may be considered established that the Papilionaceæ can take the whole of their nitrogen from the air.

The experiments of the preceding year had shown that the peas did not derive their nitrogen from the small quantity of combined nitrogen in the air, and new experiments fully confirmed this. Following the plan of BOUSSINGAULT, they put 4 kilog. of ignited sand in a large glass balloon, added mineral constituents and a small quantity of the soil-extract, and then sowed one seed of oats, one of buckwheat, and one of peas. The vessel was then perfectly closed by a well-ground glass stopper; but carbonic acid was occasionally supplied. The oats and buckwheat only grew so long as the supply of nitrogen of the seed lasted; but the peas continued to grow luxuriantly and quite normally. A large part of the produce was found to contain 6·55 grams dry substance, and 0·137 gram nitrogen.

The author says that it cannot yet be with certainty explained in what way the soil-extract enables the Papilionaceæ to assimilate the nitrogen, and that it is even doubtful whether the root-nodules have any connection with the taking up of the nitrogen. It is, however, proved that the soil-extract favours the development of the nodules, whilst the sterilised extract has no such effect. It seems natural to attribute the action to bacteria, and to connect it with the organisms in the nodules, but the experiments do not as yet settle the question.

The amount of nitrogen in the seed is not given, but to show how considerable the assimilation of nitrogen may be, the following results, showing the amounts of dry substance, and of nitrogen, in the produce of a number of the pots of lupins, are quoted :—

	Without soil-extract.			With soil-extract.	
	Dry matter.	Nitrogen.		Dry matter.	Nitrogen.
Nos.	grams.	grams.	Nos.	grams.	grams.
9	0·918	0·0146	3	44·73	1·099
10	0·800	0·0136	4	45·62	1·156
11	0·921	0·0132	5	44·48	1·194
12	1·021	0·0133	6	42·45	1·337

Such is the brief account of the experiments as yet published by Dr. WILFARTH ; and that full confidence was placed in the results by those present may be inferred, since the report states that the communication was received with great applause ; whilst in the discussion which followed, Drs. NOBBE, HEIDEN, LIEBSCHER, FLEISCHER, and EMIL VON WOLFF, took part.

It will be seen that the results are not only confirmatory of those given by HELLRIEGEL the year before, but that they are even much more definite and striking. Thus, taking no account of the fraction of a milligram of combined nitrogen supplied in the soil-extract, the amount of dry matter produced is nearly 50 times, and the amount of nitrogen assimilated is nearly 100 times, as much with, as without, the soil-extract!

The negative result with Gramineæ, Chenopodiaceæ, Cruciferæ, and Polygoneæ, is certainly just what would be expected from all that is known of the influence of soil-supplies of nitrogen on the growth of the agricultural representatives of those families. It will be observed, however, that whilst with oats and buckwheat as representatives of the Gramineæ and the Polygoneæ, HELLRIEGEL and WILFARTH got negative results, it was chiefly with rye-grass and buckwheat that M. JOULIE obtained such great gains, though it is true under very different conditions as to soil-supplies of nitrogen, whilst some of his greater gains were largely in the soil as well as in the plants.

But whilst experience, whether practical or experimental, does not point to an unsolved problem in the matter of the sources of the nitrogen of the agricultural plants of the families above enumerated, it is far otherwise so far as the Papilionaceæ are concerned. It is true that, besides other evidence, our own results, recorded in this and former papers, show that even these plants do avail themselves of nitrogen existing as nitrates within the soil; and HELLRIEGEL also distinctly recognises that such is the case. At the same time, in reference to our own experiments we have admitted that the evidence adduced does not justify the conclusion that nitrates within the soil were an adequate source of the whole of the nitrogen that was taken up in some of the cases cited. Indeed, although the question of the sources of the nitrogen of the Leguminosæ has been the subject of experiment and of controversy for about half a century, it is generally admitted that all the evidence that has been acquired on lines of inquiry until recently followed have failed to solve the problem conclusively. It should not, therefore, excite surprise that any new light should come from a new line of inquiry. Hence should be recognised, whether as real advance in knowledge, or as only incentive to further investigation, the importance of the cumulative evidence of the last few years—of which that furnished by the experiments of HELLRIEGEL and WILFARTH is certainly the most definite and the most striking, pointing to the conclusion that although chlorophyllous plants may not directly utilise the free nitrogen of the air, some of them, at any rate, may acquire nitrogen brought into combination under the influence of lower organisms, the development of which is, apparently, in some cases a coincident of the growth of the higher plant whose nutrition they are to serve.

Such a conclusion is, however, of such fundamental, and of such far-reaching importance, that further proof must yet be demanded, before it can be accepted as beyond question. Should it be eventually fully established, it would certainly suffice

to explain facts hitherto not fully explained. On the other hand, should it not be established, and a soil source of the whole of the nitrogen of the Leguminosæ be conclusively proved, the facts of agricultural production would, it seems to us, be equally well explained. To this point we shall refer again in our general concluding observations.

7. *The Experiments of Professor* EMIL VON WOLFF.

It was also at the Berlin meeting in 1886, at which one of ourselves was present, that Professor VON WOLFF distributed a page of tabulated results of vegetation experiments, made at Hohenheim, and gave some account of them. A preliminary series had been made in 1883, and more careful series were conducted in 1884, 1885, and 1886. Three sets of experiments, A., B., and C., were made, as follows :—

A. In wooden boxes, 14·5 cm. (= 5·7 inches) diameter, and 28 cm. (= 11·0 inches) deep. Into each was put 8 kilog. (= 17·6 lbs.) of calcareous coarse-grained river-sand, from which the finest part had been removed by washing. The experimental plants were—oats, sand-peas, field-beans, and red clover; and each of these was grown under the following conditions :—

1.—Without manure.

2.—With mineral manure, comprising—superphosphate, magnesium sulphate, calcium carbonate, and potassium bicarbonate.

3.—With the mineral manure, and potassium nitrate = 0·208 gram nitrogen.

4.—With the mineral manure, and potassium nitrate = 0·832 gram nitrogen.

B. In sheet zinc vessels, 25 cm. (= 9·8 inches) diameter, and 35 cm. (= 13·8 inches) deep; into each of which was put 24 kilog. (= 52·9 lbs.) of the washed river-sand. These experiments were only made in 1885 and 1886. The plants were oats, and sand-peas; and the same four conditions as to manuring, as above described, were adopted; but the quantities used were larger, the amount of nitrogen supplied in Experiment 3 being 0·416 gram, and Experiment 4—1·664 gram.

C. In cement vessels, 50 cm. (= 19·7 inches) diameter and 60 cm. (= 23·6 inches) deep. Into each was put 210 kilog. (= 463 lbs.), of the raw unwashed river-sand. The conditions of manuring were in kind the same as in A. and B.; but the quantities of nitrogen supplied were, in Experiment 3—0·832 gram, and in Experiment 4—3·328 grams. These experiments were made in 1884, 1885, and 1886; and the plants were—oats, field-beans, clover, and potatoes.

At the Berlin meeting in 1886, Professor VON WOLFF gave only the amounts of air-dried above-ground produce; but he has since published, in conjunction with Dr. C. KREUZHAGE, a long paper, giving a great deal of analytical detail.* It is there explained that, for the experiments in wooden boxes (A.), and in zinc vessels (B.), fresh washed river-sand was put in each year; but that, for those in the cement vessels (C.), the unwashed river-sand first put in was not renewed.

* "Vegetationsversuche in Sandkultur über das Verhalten verschiedener Pflanzen gegen die Zufuhr von Salpeterstickstoff." 'Landwirthschaftliche Jahrbücher,' vol. 16, Heft 4, 1887, pp. 659 *et seq.*

As to the results, WOLFF called attention to the fact that with the oats and the potatoes comparatively little increase was obtained by the use of mineral manure without nitrogen, but that where nitrogenous manure was added the increase bore a direct relation to the amount of nitric-nitrogen supplied. The behaviour of the Leguminosæ was, however, quite different. With these plants, the mineral manures as a rule gave considerable increase, whilst the addition of the nitrate generally gave little or no further increase. He remarks that these results are consistent with those obtained in ordinary agriculture; and that it is a question whether the so-called "nitrogen collectors" obtain all their nitrogen by means of their widely and deeply penetrating roots, or whether they draw some of it from the air; and if so, whether they can only take it as combined nitrogen, or also as free nitrogen? He considers that the results of BOUSSINGAULT and ourselves are against the supposition that they assimilate free nitrogen. At the same time he thinks the results of HELLRIEGEL show that the Papilionaceæ are not dependent on soil sources of nitrogen alone; though further evidence is required to determine whether or not the free nitrogen of the air comes into play.

As to the connection of the root-nodules with the development of the plants, WOLFF considers that they may be equally well supposed to be a consequence as a cause of active growth. Observations by SCHULTZ-LUPITZ, and at Hohenheim, have shown that the nodules may be very little developed in a soil rich in nitrogen. He refers to the results of FRANK and BRUNCHORST, as indicating, that the contents of the nodules do not consist either of bacteria or of fungoid forms,* but rather of nitrogenous matters which are re-absorbed by the plant when forming fruit.

As bearing on the subject, he quotes the following results of TROSCHKE at Regenwalde, showing the comparative composition of the nodules and of the roots of blue lupins, at the time of pod formation:—

	Pure ash.	Crude fat.	Crude fibre.	Crude protein.	Non-nitrogenous extract.	Nitrogen.	Of total nitrogen albuminoid.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Nodules . . .	7·51	5·33	9·43	45·31	32·42	7·25	69·7
Roots	4·07	1·31	52·25	7·06	34·61	1·13	73·7

At Hohenheim, the nodules of yellow lupins were found to be less nitrogenous at the conclusion of pod-formation.

WOLFF considers that numerous results show that atmospheric sources of nitrogen do come into play in the growth of the Papilionaceæ. He quotes from BOUSSINGAULT in reference to experiments with clover and peas grown in a nitrogen-free soil that

* It may be observed that the recent results of Professor MARSHALL WARD ('Phil. Trans.,' B, vol. 178, 1887, pp. 539–562) are at variance with the views of FRANK, BRUNCHORST, and TSCHIRCH on this point.

the plants had gained nitrogen. It is to be observed, however, that the results in question were those of BOUSSINGAULT'S earliest experiments, made in 1837 and 1838, whilst his later results with Leguminosæ obtained under similar conditions, either show slight loss or scarcely appreciable gain. WOLFF also quotes experiments of his own with clover, made in 1853, in one case in good soil, and in another in the same soil previously ignited, when the dry substance of 4 cuttings was, from the natural soil 3·396 grams, but from the ignited soil 20·314 grams; these quantities being exclusive of stubble and roots.

Referring to his more recent experiments, considerable detail is given as to the preliminary series made in 1883, as well as to the more complete series conducted in 1884, 1885, and 1886, the general conditions of which have been described above, and we now confine attention to some further account of the results obtained in the later years.

WOLFF states that the washing of the river-sand removed 1·46 per cent. of it, which consisted of sandy clayey matter. He gives a complete mineral analysis of both the crude sand, and the separated fine matter. He states that the washed sand contained only traces of nitrogen. The separated fine matter contained, however, according to a direct determination, 0·304 per cent. of nitrogen, which seemed to exist in humus compounds. It may be observed, that it was, therefore, about twice as rich in nitrogen as most ordinary arable surface soils. The results obtained in the first year 1884, in the cement vessels, with the unwashed river-sand, show the influence of this supply; but it was concluded that in 1885, and 1886, but little remained in a condition available to the plants.

All the experiments were equally exposed to the influences of air and light, excepting that the wooden boxes were placed on a low truck, which was pushed under shelter when there was violent or continued rain. All the plants were watered with distilled water when there was not sufficient rain; and in all cases the drainage was collected and used for re-watering. The sowing of the seeds was always at the end of April; the gathering of the oats, beans, and lupins, at the end of July; and the last cuttings of the clover in August and September.

To the amounts of above-ground produce (air dried), WOLFF now adds the quantity of roots, and the total weight including them. He also gives, for the produce of 1886, the amounts of dry matter, and both the percentages of nitrogen in the dry matter, and the actual quantities of nitrogen in the crops, in the case of the "C" series of experiments, that is, those made in the cement vessels, with the unwashed river-sand. In the case of the sand-peas, however, the results of the "B" series, that is, those made in zinc vessels, with the washed river-sand, are given. The following is a summary of the results, so far as the nitrogen is concerned:—

Total Nitrogen in the Crops of 1886.

	"C" series, unwashed sand.				"B" series, washed sand.
	Oats.	Lupins.	Field-beans.	Red clover.	Sand-peas.
	grams.	grams.	grams.	grams.	grams.
1. Unmanured	0·371	2·797	2·478	3·396	0·488
2. Mineral manure	0·388	9·572	4·689	8·352	2·389
3. Mineral manure, and 0·832 gram N	1·136	5·392	3·232	8·803	3·698
4. Mineral manure, and 3·328 grams N	3·486	8·816	4·104	9·403	5·069

It should be observed that the quantities of nitrogen in manure, as stated in the table, relate to the "C" Series in unwashed sand, but the quantities supplied to the smaller vessels of the "B" Series for the sand-peas were for Experiment 3, 0·416 gram, and for Experiment 4, 1·664 gram. On the results, WOLFF remarks that without exception the cereal crop (oats) only flourished when there was a sufficient quantity of nitrogenous nutriment (nitric-nitrogen) available; whilst the Leguminosæ for the most part grew quite as luxuriantly without, or with very little, nitrogen in the soil, provided the ash constituents were in abundance. Potatoes, on the other hand, like the oats, required combined nitrogen to be provided within the soil.

The mineral composition of the crops, and the influence of the varying climatal conditions of the three seasons, are discussed in some detail. Estimates are given of the amounts of phosphoric acid, potash, &c., in the crops, calculated per hectare; and the quantity of nitrogen in the oat crop is estimated to correspond, in one case, to 139·4 kilog. per hectare (= 124·5 lbs. per acre), which is very large.

Excluding the experiments in the unwashed sand, the mean of 7 experiments under each condition of manuring with oats, and of 22 under each condition with different Leguminosæ, shows the following amounts of air-dried produce :—

	Oats. Mean of 7 experiments in each case.	Leguminosæ. Mean of 22 experiments in each case.
	grams.	grams.
1. Unmanured	16·87	22·71
2. Mineral manure	18·31	68·53
3. Mineral manure, and 0·238 gram nitrogen for the oats, and 0·180 gram for the Leguminosæ	47·42	73·39
4. Mineral manure, and 0·952 gram nitrogen for the oats, and 0·72 gram for the Leguminosæ	110·74	74·95

The little influence of the mineral, and the great influence of the nitrogenous manure, on the oats, and again the marked influence of the mineral, and the little effect of the nitrogenous manure, on the Leguminosæ, are here very strikingly illustrated.

Referring to the amounts of nitrogen in the crops of sand-peas grown in the washed sand, as given in the table at the top of page 74, WOLFF states that the 19 peas sown contained 1·542 gram dry substance, and he estimates the amount of nitrogen in the seed sown at only 0·0647 gram; the quantity of nitrogen so provided was, therefore, quite immaterial in proportion to that in the crops.

The amounts of nitrogen in the clover crops of Experiments 2 and 4 are estimated to correspond to 334 and 376 kilog. per hectare, to which one-third should be added for stubble and roots. The beans appear not to have collected so large a quantity of nitrogen in 1886 as the clover and the lupins; and it may be mentioned that whilst the 54 bean seeds sown would contain 1·0353 gram of nitrogen, the same number of lupin seeds would contain only 0·5912 gram.

Some experiments by STRECKER with yellow lupins are quoted as showing considerable gains of nitrogen.

With regard to the numerous results recorded in his paper, WOLFF admits that they are not satisfactory in all respects, those of different years not being always accordant. But the special object was to compare the growth of cereals with that of Papilionaceæ, over a series of years, in a soil poor in combined nitrogen, or containing known amounts of it; and the influence of the various seasons was different on the different crops.

The following table gives estimates of the amounts of nitrogen in the crops, more (+) or less (−) than supplied in the seed and manure, calculated per hectare; the selection of experiments being the same as in the case of the results given in the table at the top of p. 74:—

Estimated Nitrogen, in kilograms per hectare, in crops more (+) or less (−) than in seed and manure.

	"C" series, unwashed sand.				"B" series washed sand.
	Oats.	Lupins.	Field-beans.	Red clover.	Sand-peas.
	Kilog.	Kilog.	Kilog.	Kilog.	Kilog.
1. Unmanured	− 9·0	+ 88·3	+ 57·7	+ 135·8	+ 84·7
2. Mineral manure	− 8·3	+ 359·3	+ 146·2	+ 334·1	+ 464·9
3. Mineral manure, and nitrate	− 11·7	+ 158·8	+ 54·6	+ 318·8	+ 643·5
4. Mineral manure, and nitrate	− 17·6	+ 195·8	− 10·4*	+ 242·9	+ 668·1

* Given in error as + 89·5 by WOLFF, the nitrogen of the manure of Experiment 3 instead of that of Experiment 4 having been deducted.

It is added that these amounts do not include the nitrogen in the stubble and roots, which in the case of the oats would be very little, in that of the beans and lupins not much, but in the sand-peas considerable, as also in the clover. It is to be observed, moreover, that no account of the nitrogen in the unwashed sand is here taken. But WOLFF points out that the gain was the largest in the case of the peas grown in the washed sand, which showed only a trace of combined nitrogen. Again the cement cases and their contents were exposed to the weather from year to year whilst there was no crop; but WOLFF points out, as is doubtless true, that the amount of combined nitrogen brought down in the rain and dew would be quite immaterial.

Admitting it to be established that plants do not assimilate the free nitrogen of the air, he thinks the only remaining hypothesis is that certain plants are enabled to appropriate the combined nitrogen of the air, either directly through their leaves, or by absorption in the soil; and the latter he considers by far the most probable. In reference to this point he refers to the results of experiments made to determine the amount of ammonia absorbed from the air by dilute acids exposed in shallow vessels. In this way A. MÜLLER estimated that 12 kilog. of ammonia were absorbed per hectare (= 10·7 lbs. per acre), per annum, in Sweden; whilst O. KELLNER'S estimate in Japan, was 14 kilog. per hectare (= 12·5 lbs. per acre). O. KELLNER also determined the amounts of nitric and nitrous acids absorbed by solutions of potassium carbonate. The quantities of nitrogen so absorbed corresponded to 11·78 kilog. per hectare as ammonia, and to 1·30 kilog. as nitric and nitrous acids; giving a total of 13·08 kilog. of nitrogen per hectare (= 11·68 lbs. per acre), per annum. Other experimenters have, however, found much more. Thus, reckoning according to SCHLŒSING'S experiments, in one of which a quantity of soil gained at the rate of 2·59 kilog. ammonia per hectare in 14 days, and in another 4·097 kilog. in 28 days, the amounts absorbed would be in the one case 68, and in the other 53 kilog. per hectare (= 60·7 and 47·3 lbs. per acre) per annum. WOLFF points out that even these amounts are small compared with the quantities of nitrogen assimilated in the experimental crops. He further remarks that his porous sand would probably present more than a hundred times the absorbing surface of an acid or alkaline solution of the same area.

Besides ammonia absorption, he thinks there is probably another way in which a humus-free soil may become a source of nitrogen to plants; viz., by the combination of free nitrogen, under the influence of calcium carbonate. He quotes B. FRANK ('Landw. Presse,' March 2, 1887), as having shown that a marl rich in lime, with which SCHULTZ-LUPITZ marled his soil, constantly yielded nitric acid after being boiled out with water, by which it is supposed it would be sterilised; and this was the case when the mass was exposed to ammonia-free air. According to the results obtained, a kilogram of the mass would acquire nearly a gram of nitric acid per annum. Pure calcium carbonate acted in a similar manner. After being washed out with hot water on a large filter, and kept moist, but protected from dust, nitrification took place. FRANK considers the calcium carbonate of the soil as a nitrogen combiner; and that,

in presence of water and atmospheric air, nitrite and nitrate of lime are gradually formed, the nitrogen and oxygen of the air uniting in contact with the porous body, and the acid uniting with the lime and expelling carbonic acid.*

WOLFF further quotes the observations of CLOËZ, made more than 30 years ago, in which he passed air, first through a solution of potassium carbonate, then through sulphuric acid and over pumice moistened with sulphuric acid, for six months over various porous substances. He found a formation of nitric acid when the air was passed over pieces of brick or pumice moistened with potassium carbonate; and also traces with chalk, chalk marl, and a mixture of kaolin and calcium carbonate. On the other hand he found no formation of nitric acid by burnt bones moistened with potassium carbonate, or by clay.

WOLFF considers that the conditions of his experiments involved those found by CLOËZ to favour such formation of nitric acid. He admits, however, that it is difficult to explain why the action should take place when the Leguminosæ are present, and that the growth of the cereals is not benefited thereby. He suggests whether the greater pumping action of the leaves of the Leguminosæ causes a more active aëration of the soil, and so it may be that with their increased development the greater is the amount of nitrogenons nutriment accumulated from the atmosphere by the moist soil, whilst it is well known that these plants leave an efficient nitrogenous residue for succeeding crops.

In conclusion, WOLFF admits that the amounts of absorption indicated in the experiments with particular plants cannot be expected on a large scale. In practice, soils are not kept so porous, and so constantly moist; nor are the mineral conditions of the soil always so favourable. Indeed the variations of result in the different experiments illustrate the influence of varying conditions.

Perhaps the most striking of WOLFF's results were those obtained in the experiments made in 1853, in which clover yielded about six times as much dry produce grown in an ignited rich meadow soil, as in the same soil in its natural state. The ignited soil would not only be nitrogen free, but sterilised; so that, unless it acquired and developed micro-organisms during the growth, the supposition of the intervention of such agents in bringing free nitrogen into combination within the soil would be excluded. In reference to this point it may be remarked, that in the case of HELLRIEGEL's experiments, in which he added the watery extract from various soils to his quartz sand, he states that red clover showed no special result.

Next as to WOLFF's more recent results, in which river-sand was used as soil, in some cases unwashed, but in others washed free from the fine matter which contained nitrogen. It is, as he says, quite consistent with experience in agriculture, that oats and potatoes should yield little increase by mineral manure without nitrogen, but give increase much in proportion to the nitrogen supplied to the soil; and that, on the

* The above results of FRANK have since been called in question by H. PLATH ('Jahrbücher,' vol. 16, 1887, p. 891, and vol. 17, 1888, p. 725); also by Professor LANDOLT, 'Landw. Presse,' Jahrgang 15, no. 30.

other hand, the Papilionaceæ should give marked increase with mineral manure, and but little with nitrogenous manure. Such is certainly the result in ordinary soils containing nitrogen; but if we are to assume that in his experiments the sand was not the source of the nitrogen, both the amounts of dry produce, and those of nitrogen, in the different crops, as shown in the tables which have been given, are such as seem to exclude any other explanation than that the air had contributed nitrogen in some way.

At the same time, the conditions of experiments conducted in a not absolutely nitrogen-free soil, and with free exposure to the weather, and so subject to accidental sources of more or less combined nitrogen which such conditions necessarily imply, however appropriate for obtaining initiative results and general indications, seem scarcely suitable for the settlement of so delicate a question as that of the source of the nitrogen of vegetation. On this point it may be remarked that, according to the data given, the unwashed sand put into each cement vessel would contain about 9 grams of combined nitrogen, whilst, as shown in the table at page 74, the largest crops of the lupins and red clover, with mineral but without nitrogenous manure, only contained about that amount of nitrogen, and the beans only about half as much. It is true that this was the result in the third year, 1886, after somewhat similar amounts had already been grown for two years.

Again, so far as the sand did contain nitrogen, the great difference of result with the Gramineæ and the Leguminosæ, under the influence of mineral manure without nitrogen, is not absolutely conclusive evidence that the Leguminosæ had acquired nitrogen from some other source than the soil; for there can be little doubt that Leguminosæ do utilise nitrogen existing in the soil in a condition in which it is not available to the Gramineæ. The results obtained in the washed sand, must, however, be admitted to have much greater significance.

As to the explanation of the results, WOLFF is disposed to attribute the gains of nitrogen to the absorption of combined nitrogen from the air by the soils, and to the fixation of free nitrogen within the soil under the influence of porous and alkaline bodies, as supposed by CLOËZ and FRANK, rather than to the fixation of free nitrogen either under the influence of micro-organisms, or directly by the plants themselves. In fact, neither were the conditions of his experiment with the clover in burnt soil, nor those of his later experiments in washed sand, such as to favour the supposition of the intervention of micro-organisms. On the other hand, he considers they were favourable for the absorption of combined nitrogen from the air, and for the supposed fixation of free nitrogen within the soil under the influence of porous and alkaline bodies. At the same time, he admits that it is not easy to explain why Gramineæ do not, equally with the Leguminosæ, benefit by such absorption, and by such fixation.

For our part we believe that a careful consideration of all that is involved in this undoubted fact, points to the exclusion of the supposition that the gain is either by the absorption of ammonia from the air, or by the fixation of free nitrogen within the

soil under the influence of porous and alkaline bodies. We shall refer to the point again in our concluding remarks, but we may here say in passing, that the results of our experiments on the growth of wheat for many years in succession on the same land without nitrogenous manure, show that with much less nitrogen annually removed in the crops, and estimated to be lost by drainage, than would be required for the growth of the Leguminosæ in WOLFF's experiments, the soil has nevertheless lost much nitrogen. Again, taking the average of ten years, the amount of nitrogen as nitric acid which has passed through 60 inches depth of soil and subsoil in the Rothamsted drain-gauges, exposed to the air and rain, with aëration from below also, but without vegetation, has been somewhat less than 40 lbs. per acre (= 44·8 kilog. per hectare) annually; and it cannot be doubted that at least the greater part of this has been derived from the organic nitrogen of the soil and subsoil. It is obvious, therefore, that the amount due to absorption and to such fixation together, must be much less than this.

If, therefore, neither nitrogenous impurity in the sand, nor accidental sources of combined nitrogen, can be supposed to account for the gains by the Leguminosæ in WOLFF's experiments, it would seem that the explanation must be sought, either in the agency of micro-organisms, or in direct assimilation by the plants themselves.

8. *The Experiments of Professor* W. O. ATWATER.

Professor ATWATER, of the Wesleyan University, Middletown, Conn., U.S.A., has published three papers in the 'American Chemical Journal':—1. "On the Acquisition of Atmospheric Nitrogen by Plants" (vol. 6, No. 6); 2 (with E. W. ROCKWOOD). "On the Loss of Nitrogen by Plants during Germination and Growth" (vol. 8, No. 5); 3. "On the Liberation of Nitrogen from its Compounds and the Acquisition of Atmospheric Nitrogen by Plants" (vol. 8, No. 5). In these papers he gives the results of experiments of his own, and discusses the results of others also, on various aspects of the question. In what years his experiments were made is not stated, but we assume in 1883 and 1884, as they were undertaken after a visit he paid to Europe in 1882, and in the autumn of 1884 he gave a paper on the subject, at the meeting of the British Association at Montreal, and also at the meeting of the American Association at Philadelphia.

It is stated that the question was :—"*May plants, grown under normal conditions, acquire any considerable amount of nitrogen, free or combined, from the ambient atmosphere?*" It is further stated, that after a series of trials had shown a not inconsiderable acquisition of atmospheric nitrogen, a second series was planned to verify the results of the first, and to include a collateral inquiry, namely :—

"*How is the acquisition of nitrogen from the atmosphere affected by abnormal conditions of growth, and what bearing may the results obtained have upon the interpretation of those obtained by other experimenters, and upon the general question of the assimilation of atmospheric nitrogen by plants?*"

Professor ATWATER first made two series of experiments in which he grew peas in sand, to which he supplied nutritive solutions, containing mineral matters, and known quantities of nitrogen as nitrate. The plants were grown in free air, but protected from rain and dew.

The following are the actual amounts of nitrogen supplied in seed and nutritive solution, the actual gains, and the gains per cent. on the total amounts supplied, in the first series of 3 experiments :—

Supplied . .	millig. .	103·7	120·6	95·7
Gains . . .	millig. .	+ 63·5	+ 13·2	+ 13·0
Gains . . .	per cent.	+ 61·2	+ 10·9	+ 13·6

The second series included 12 experiments, in six of which generally less, and in the other six generally much more, nitrogen was supplied in the solution than in the seed.

With the smaller quantities of total nitrogen the results were :—

Supplied .	millig. .	94·7	128·2	93·6	130·9	93·3	129·2
Gains . .	millig. .	+ 21·7	+ 30·7	+ 62·5	+ 27·2	+ 93·2	+ 81·7
Gains . .	per cent.	+ 22·9	+ 23·9	+ 66·8	+ 20·8	+ 99·9	+ 63·2

With the larger quantities of nitrogen the results were :—

Supplied . .	millig. .	169·3	199·3	170·5	194·5	135·8	161·0
Gains (or loss)	millig. .	+ 9·6	+ 1·3	− 20·9	+ 3·0	+ 142·0	+ 99·2
Gains (or loss)	per cent.	+ 5·6	+ 5·7	− 12·3	+ 1·5	+ 104·5	+ 62·0

With regard to the variations of result, it is concluded that where the gains were less, or there was loss, the conditions were abnormal, and where the conditions were normal, the gains were the higher.

Some of the results are calculated per hectare and per acre, showing very large gains compared with the amounts of nitrogen in ordinary crops in the field.

Numerous experiments were also made to determine the loss of nitrogen in the germination of peas ; five by the water-culture method, and eight in sand.

Three of the water-culture experiments were conducted in open air, when the results were :—

Nitrogen in seed .	millig. .	215·6	67·5	41·4
Nitrogen lost . .	millig. .	− 18·1	− 6·6	− 0·6
Loss	per cent.	− 8·4	− 9·8	− 1·5

In two water-culture experiments in the air of the laboratory, there was gain not loss of nitrogen, which was attributed to ammonia in the air. The results were :—

In seed . .	millig. .	290·2	119·7
Gains . . .	millig. .	+ 19·2	+ 10·2
Gains . . .	per cent.	+ 6·6	+ 8·5

Of the 8 germination experiments in sand, 3 were conducted in the open air. The results were :—

In seed . .	millig. .	100·3	102·9	109·7
Loss . . .	millig. .	− 6·0	− 10·9	− 16·6
Loss . . .	per cent.	− 5·9	− 10·6	− 15·1

Three experiments in a greenhouse showed :—

In seed . .	millig. .	115·6	103·4	101·4
Loss . . .	millig. .	− 8·4	− 12·7	− 16·5
Loss . . .	per cent.	− 7·3	− 12·4	− 16·3

Lastly, two experiments in a room in a dwelling-house, showed :—

In seed . .	millig. .	88·3	88·2
Loss . . .	millig. .	− 7·2	− 9·7
Loss . . .	per cent.	− 8·2	− 11·0

ATWATER discusses the results of other experimenters, including those of BOUSSINGAULT, SCHLŒSING, and ourselves and the late Dr. PUGH, on the evolution of free nitrogen under various conditions. On the whole he concludes, that germination without the liberation of nitrogen is the normal process; that losses, whether during germination, or in later periods of growth, are due to forms of decay; and that they would thus be not essential to germination and growth, but accessory phenomena. He, nevertheless, gives a table showing, for 8 of his experiments, the actual gains found, the greater gains supposing there had been a loss of 15 per cent. of nitrogen—as in some of his own germination experiments, and the still greater gains supposing a loss of 45 per cent., as shown in experiments of BOUSSINGAULT in the germination of beans and maize, under special conditions, in contact with nitrate. He admits, however, that there is no evidence to show what was the loss in his own vegetation experiments.

We may here remark, that, in full recognition of the loss of nitrogen under conditions of decay, it was concluded, both by BOUSSINGAULT and at Rothamsted, that excepting in some cases in which there was obviously some decay, the results were not vitiated by any such loss, and in those cases the losses found were so explained.

ATWATER quotes BOUSSINGAULT'S earliest experiments, made in 1837 and 1838, to show that with clover and peas nitrogen must have been gained from the air. It

should be remarked, however, that BOUSSINGAULT himself, after he had acquired much more experience, both in the conduct of vegetation experiments, and in analytical method, entirely disallowed that the results of those early experiments were evidence of the fixation of free nitrogen. He also quotes BOUSSINGAULT'S later experiments; made in 1851, 1853, and 1854, and says in regard to them that—"even the cereals, oats and wheat, contain 17 to 32 per cent. more than was supplied in the seed. The results differ from those of the previous series in that the cereals here show more, and the legumes less, gain of nitrogen." Now, in the 6 cases with haricots and lupins referred to, the amounts of nitrogen supplied in the seed ranged from 19·9 to 36·7 millig., and they show, respectively—2·3 millig. actual loss, and 3·2, 2·5, 4·2, 3·0 and 2·0 millig. actual gain. In the case of the cereals, on the other hand, the total amount of nitrogen supplied in the seed was, with the oats only 3·1, and with the wheat only 6·4 millig., and the actual gains shown were only 1·0 and 1·1 millig. Yet, ATWATER shows that these last results, calculated into *percentage*, represent gains of 32 and 17 per cent. of the original nitrogen, and larger gains even than with the legumes! It need hardly be said, that BOUSSINGAULT interpreted these later results as not indicating any fixation of nitrogen.

Briefly summarised, ATWATER'S conclusions are:—

1.—That in some of his experiments with peas, half or more of the total nitrogen of the plants was acquired from the air. Where the gains were small, or there was a loss, the conditions were abnormal, and it is to be assumed that there was loss, either from the nitrate of the nutritive solutions, from the seeds during germination, or from the growing plants.

2.—An actually observed gain is positive proof that nitrogen has been assimilated, either directly by the plants, or indirectly through the medium in which the roots have developed. The failure of an experiment to show gain only proves non-assimilation, if it is also proved that there was no liberation of nitrogen. The conflicting results of various experimenters may probably be explained by the fact of such liberation.

3.—The experiments do not show in what way the nitrogen is acquired. It must have been taken up, either as free or combined nitrogen, either directly through the foliage, or indirectly through the soil and nutritive solutions, and the roots.

4.—It is possible that the negative results of BOUSSINGAULT, and ourselves, are due to the liberation of free nitrogen. The conditions were, moreover, such as to exclude the action of electricity and of microbes.

5.—Since BERTHELOT has shown that nitrogen may be fixed in organic matter by the agency of electricity, and in soils by the agency of micro-organisms, some cases of gain may be so explained; but it is considered that the conditions for such actions did not exist in his own experiments. The balance of evidence favours the assumption that the plants themselves were the agents.

6.—The conclusion that plants acquire atmospheric nitrogen accords with, and

explains, facts of vegetable production otherwise unexplained; and the fact of its acquisition in considerable quantities seems well established.

We have pointed out that in BERTHELOT'S later papers he seems to rely much more on the agency of micro-organisms, than on that of electricity, in explanation of the phenomena of the fixation of free nitrogen; whilst ATWATER does not consider that the conditions of his own experiments are favourable to the supposition that either of these agencies was the cause of the fixation which his results show.

It need only be added, that the assumption that the real gains are generally greater than the experimental results indicate, on account of the losses that have taken place, is a very old one, it having been brought against the negative results obtained by BOUSSINGAULT, and at Rothamsted, thirty or more years ago. It is still, however, as has been seen, a favourite argument with others as well as Professor ATWATER. We have already said, that neither the conclusions of BOUSSINGAULT, nor those drawn from the Rothamsted experiments, were vitiated by virtue of such loss. Further, the supposition that the assimilation of free nitrogen is the greater when luxuriance is favoured to a certain degree by artificial supplies of combined nitrogen, owes its origin to about the same date. The result was, however, as distinctly negative in the experiments at Rothamsted when luxuriance was so favoured, as when it was not. It is freely admitted, however, that in many of the experiments of BOUSSINGAULT, as in those at Rothamsted, the arrangements were such as to exclude the agency, either of electricity or of micro-organisms. To this point we shall refer again presently.

9. *Recent results and conclusions of* M. BOUSSINGAULT.

We have frequently discussed the results of M. BOUSSINGAULT, obtained from 1837 to 1854, and expressed entire agreement with the conclusions he drew from them under the conditions provided; and it is not the object of the present comments to reconsider them in any detail. The question of the fixation of free nitrogen has, however, assumed a new aspect in recent years. It is now supposed that fixation, either by the plant, or within the soil, takes place, if at all, by the agency of electricity, or of micro-organisms, or of both; and there can be no doubt, that the earlier vegetation experiments of BOUSSINGAULT above referred to, as well as those conducted at Rothamsted about thirty years ago, were so arranged as to exclude the influence of either of those agencies. If, therefore, it should be established, that fixation does take place under their influence, and that such influence is essential for the development of the action, the conclusions, both of BOUSSINGAULT and ourselves, from the results in question, are so far vitiated. It is to some of BOUSSINGAULT'S more recent results, which have a bearing on this new aspect of the question, that we propose now to refer.

In BOUSSINGAULT'S previous vegetation experiments he had used sterilised materials as soils. But, in 1858,* he commenced a series in which he employed more or less of a

* 'Comptes Rendus,' vol. 48, 1859, p. 303. 'Agronomie, &c.,' 2e édit., vol. 1, 1860, p. 283.

rich garden surface soil, mixed with more or less sand, or quartz, or both. The plants grown were lupins, hemp, and haricots; in some cases in free, and in others in confined air. In the latter cases, the materials were put into a large glass balloon, or carboy, moistened with pure distilled water, the seed sown, and then the whole perfectly closed from the outer air by means of caoutchouc, arrangement being made, however, for the supply of carbonic acid.

In the experiment with lupins in confined air, the largest amount of the rich soil was used, and the result was so striking, that BOUSSINGAULT repeated it the next year, 1859,* when he obtained an almost identical result. In no other case was there anything like the same amount of gain of nitrogen, and we must only refer in any detail to the conditions and the results of these two experiments. They were as follows:—

Date.	Plant.	Air.	Soil, &c.				Nitrogen.						
							At commencement.			At conclusion.			
			Soil.	Sand.	Quartz.	Ash.	In soil.	In seed.	Total.	In soil.	In plant.	Total.	Gain.
			grams.	grams.	grams.	grams.	grams.	grams.	grams.	grams.	grams.	grams.	grams.
1858	Lupin	Confined	130	1000	500	0·2	·3393	·0204	·3597	·4065	·0246	·4311	+·0714
1859	Lupin	Confined	130	720	150	0·1	·3380	·0200	·3580	·3834	·0417	·4251	+·0671

It should be stated that, taking the mean of 7 determinations by the soda-lime method, the rich garden soil used contained 0·261 per cent. of nitrogen. This BOUSSINGAULT calculated would correspond to 11,310 kilog. nitrogen per hectare (= 10,098 lbs. per acre), one-third of a metre deep. He also determined the amounts of ammonia, nitric acid, and carbon, in the soil; and he concluded that the nitrogen, beyond the small amount existing as ammonia or nitric acid, was in combination as organic matter. In fact it existed in organic detritus, and especially in a black substance which he observed by the microscope.

Referring to the figures, it is seen that in the experiment in 1858 there was a gain of 0·0714 gram nitrogen, upon a total of 0·3597 supplied in soil and seed. Further, calculation shows that, of the total gain 0·0672 gram was in the soil, and only 0·0042 gram in the plant, notwithstanding that the original soil contained 0·3393 gram nitrogen. BOUSSINGAULT remarks that the fertilising matters in the soil had thus scarcely taken any part in the growth; the conclusion being that it was only the nitrogen that existed as, or was transformed into, ammonia or nitric acid, that was available. He further remarks, that it was impossible, that anything like the amount of excess of nitrogen in the soil could be due to the débris of the vegetable matter of the lupin, roots, &c. He adds:—*It is the soil and not the plant which has fixed the nitrogen; and one such result can only be admitted if confirmed by future experiments.*

* 'Agronomie, &c.,' p. 329.

In the same year, 1858, another experiment was made with lupins, but in free air, one with hemp in free air, two with haricots, one in confined and one in free air, and one with 120 grams of the rich soil placed in a shallow vessel, kept moist with distilled water, and exposed to the free air as an experiment on fallow. In this last experiment, whilst there was a loss of about one-third of the carbon, the nitrogen increased from 0·31320 gram to 0·32184 gram, showing a gain therefore of 0·00864 gram.

Boussingault remarks that whilst the soil has lost a considerable quantity of its carbon by slow combustion, the nitrogen instead of diminishing has even increased, and that it remains to decide whether there has been nitrification, production, or simple absorption of ammonia.

In introducing the report of the second series of experiments, those made in 1859, Boussingault says that he could not accept the gain of nitrogen by vegetable mould as sufficiently established, without repeating the experiments. It further remained to examine whether, in case there really were a fixation of nitrogen, it was as nitric acid, as ammonia, or as organic compounds.

In reference to the result of the experiment made in 1859, as given in the table, Boussingault says that during the growth of a lupin in a confined atmosphere, in 130 grams of very rich soil, mixed with sand to favour the access of air, the plant, during 97 days, assimilated 0·0217 gram nitrogen from the soil, and yet the soil gained 0·0454 gram nitrogen, only one-ninth of which pre-existed as nitric acid or ammonia. The total gain of nitrogen in plant and soil was 0·0671 gram, a result which is almost identical with that found in 1858. He adds, that there is this curious coincidence, that in both cases it is by the soil, and not by the plant, that the gain has been effected.

In the case of none of the other vegetation experiments in 1859 are the gains or losses by the soils given, so that the total gain or loss cannot be estimated. Boussingault points out, however, that in 1859, there was about twice as much nitrogen taken up by a haricot growing in 100 grams of the soil, as by one growing in only 50 grams in 1858. It may be added that haricots took up much more nitrogen in proportion to a given amount of soil than lupins.

Referring to the main results, Boussingault says the singular fact appears, that the soil not only gained ammonia and nitrates, but organic matter also, possibly the remains of living organisms. On careful examination, he has observed that vegetable earth contains, not only dead organised matter, but living organisms, germs, the vitality of which is suspended by drying, and re-established under favourable conditions as to moisture and temperature. This mycodermic vegetation is not always visible to the naked eye, and its progress must be followed by the aid of the microscope. The mycoderms have only an ephemeral existence, and they leave their detritus in the soil, which in time may give rise to ammonia and nitric acid. Even if the nitrogen of the air takes part in nitrification, a part of the nitrogen will exist in mycoderms, or their remains.

However this may be, considering only the numerical results which have been obtained, he is forced to believe that the soil of Liebfrauenberg has fixed nitrogen; nitric acid and ammonia being at the same time developed. The experiment on fallow in confined air seems to indicate that the vegetation has but little to do with the result.

Having given the details of his experiments, he submits them to the criticism of others, thus enabling them to judge whether the intervention of the nitrogen of the air in the production of nitrates is really established. In his opinion, if there is not absolute proof, there is certainly strong presumption, in favour of the reality of the phenomenon.

These very remarkable results seem to have instigated new experiments to test the validity of the obvious conclusion from them. In the discussion of the previous experiments BOUSSINGAULT had constantly compared the results obtained in a vegetable soil with those in a nitre bed. In reference to these new experiments he says that in the nitrification of vegetable earth, and in the materials of an artificial nitre bed, everything leads to the conclusion that the nitric acid is developed especially at the expense of organic substances. But it does not necessarily follow that the gaseous nitrogen of the atmosphere cannot contribute, within certain limits, to the production of nitrates. It is to ascertain whether this co-operation takes place that the new experiments were undertaken.

In the next year, 1860,* BOUSSINGAULT placed a mixture of 100 grams of very rich vegetable soil, and 300 grams of quartz sand, in a large balloon, such as he used in the previous vegetation experiments, moistened the mass, and then closed it perfectly by means of a caoutchouc cap. A second experiment was also arranged, in which the conditions were precisely similar, excepting that 5 grams of cellulose were added to the mixture. The materials could not be stirred, and it was decided to leave them in contact with confined air for a considerable time. The two vessels were, in fact, left for 11 years, when, in 1871, they were opened, and the contents examined.

The result was that in both cases there was a very considerable amount of nitrification, representing in Experiment 1 rather more, but in Experiment 2 with the cellulose less, than one-third of the original nitrogen of the soil. The actual loss of carbon was more than 4 times as great in Experiment 2 with the cellulose, as in Experiment 1 without it; amounting in Experiment 1 to about 16 per cent., and in Experiment 2 to about 43 per cent. of the original quantity.

Lastly, as to the nitrogen:—In Experiment 1, without cellulose, there was out of 0·4722 gram total nitrogen in the original soil, a loss of 0·0212 gram, corresponding to 4·5 per cent. of the original amount; and in Experiment 2, with the cellulose, there was, upon the same original amount of nitrogen a loss of 0·0081 gram, corresponding to 1·71 per cent. of the original.

* 'Compt. Rend.,' vol. 76, 1873, p. 22.

In regard to these new results BOUSSINGAULT says that, contrary to his anticipation, the combustion of the carbon of the non-nitrogenous organic matter, the cellulose, added to the soil, had not favoured the production of nitric acid.

He gives reasons for concluding that the process of nitrification had been completed before the opening of the vessels in 1871. At the same time, he shows that the amount, both of oxygen, and of salifiable bases, remaining, was sufficient for the production of much more nitrate.

Upon the whole he concludes as follows :—

It results from these researches, that, in the nitrification of vegetable soil, in a confined atmosphere not renewed, that is in stagnant air, gaseous nitrogen does not appear to contribute to the formation of nitric acid. The nitrogen determined in the soil in 1871, was not more than, but was even not quite so much as, in 1860. In the conditions of the experiment, the nitrification must have taken place at the expense of the organic substance of humus, which is found in all fertile soils.

Although, as we have already said, the experiments in question were obviously suggested by the results obtained in 1858 and 1859, which showed a gain of nitrogen, BOUSSINGAULT does not, throughout the discussion of the new results, offer any explanation of, or even refer to, the earlier ones. Further, it will be observed that in recording the negative results of the new experiments, he is careful to define the conditions under which they were obtained.

Always placing the greatest reliance, both in the work and in the conclusions of BOUSSINGAULT, we had been much impressed with the significance and importance of the earlier results and conclusions above referred to, which did not seem to be satisfactorily explained by the new ones, and in April, 1876, one of us wrote to him as follows :—

"We have been very much struck with some of your results with Leguminosæ, especially those with lupins in confined air in 1858 and 1859, and those with lupins and haricots in free air in 1858. May I ask whether it is your opinion that the free azote of the air does enter into combination, either by the direct agency of vegetation, or through that of the soil? And, if so, under what conditions do you think this action takes place, and what is the nature of the action?"

In answer BOUSSINGAULT wrote a long and interesting letter, dated May 19, 1876, in which he discussed various points of the subject of the sources of the nitrogen of vegetation, and replied as follows in reference to the special questions relating to his experiments in 1858 and 1859 :—

"Quant à l'absorption de l'azote gazeux de l'air par la terre végétale je ne connais pas une seule observation irréprochable qui l'établisse ; non seulement la terre n'absorbe pas d'azote gazeux mais elle en émet, ainsi que vous l'avez reconnu avec Mr. LAWES, comme l'a vu REISET pour le fumier, comme nous l'avons constaté, M. SCHLŒSING et moi, dans nos recherches sur la nitrification.

"S'il est en physiologie un fait parfaitement démontré, c'est celui de la non assimilation de l'azote libre par les végétaux, et je puis ajouter par les plantes d'un ordre inférieur, telles que les mycodermes, les champignons."

Thus, then, although by the terms of our inquiry, BOUSSINGAULT's attention was specially directed to the evidence of gain of nitrogen from the air by the soil which his experiments in 1858 and 1859 afforded, he, in 1876 states that he is not aware of any irreproachable observation establishing the reality of such an action, whilst, on the contrary, he considers it established that soils emit rather than gain free nitrogen.

Further, he considers it perfectly demonstrated, that neither plants of a higher, nor those of an inferior order, such as mycoderms and fungi, assimilate free nitrogen.

It is to be observed that, although BOUSSINGAULT clearly ignores the significance of the results to which we had directed his attention, he did not offer any explanation of them. Subsequently, on several occasions when passing through Paris, one of us sought to meet M. BOUSSINGAULT, and to discuss the question with him further, but he was each time in Alsace. However, one of us visited him at Liebfrauenberg in 1883, and had an interesting conversation with him on the subject. No special reference was made to his experiments of 1858 and 1859; but he clearly maintained the same view as to the non-fixation of free nitrogen, as given in the sentences above quoted from his letter of 1876.

It is remarkable, that in that letter he should so expressly give his opinion against the supposition that the lower organisms within the soil effect the fixation of free nitrogen, notwithstanding the evidence of his experiments of 1858 and 1859 that the gain, if there really were gain, was chiefly by the soil, and chiefly as organic matter, the accumulation of which he attributed to the development of mycodermic vegetation. It is true that, in the discussion of the results, he did not give any clear indication whether he considered that the apparent fixation was due in the first instance to the process of nitrification, the mycoderms only appropriating the nitrogen of the nitrates formed, or whether he supposed that the mycoderms themselves were the primary agents, and that the nitrification was only the result of the oxidation of the mycodermic remains.

It did indeed seem, that, in the results in question, there was *the germ of the germ explanation* of the fixation of free nitrogen, if such took place at all, in connection with vegetation. But we confess that BOUSSINGAULT's very distinct conclusion against the assumption of any such agency, notwithstanding the indications of some of his own experiments, leads us still to ask for further confirmation of the evidence of others in the same direction, which has been accumulating during the last few years.

PART III.

Summary, and General Considerations and Conclusions.

It seems desirable to endeavour to summarise the results, both experimental and critical, of this extended inquiry, relating to a very difficult and very complicated subject, and involving the consideration of very conflicting evidence, and of equally conflicting opinions in regard to it.

We will first give a *résumé* of the results, and conclusions, as given in Part I. of this paper, on—

1. *The Evidence relating to other Sources than Free Nitrogen.*

In our earlier papers we had concluded that, excepting the small amount of combined nitrogen coming down in rain and the minor aqueous deposits from the atmosphere, the source of the nitrogen of our crops was, substantially, the stores within the soil and subsoil, whether derived from previous accumulations, or from recent supplies by manure.

More recently, we have shown that the amount of nitrogen as nitric acid in the soil, was much less after the growth of a crop than under corresponding conditions without a crop. In the case of gramineous crops it was concluded that most if not the whole of their nitrogen was taken up as nitric acid. In the experiments with leguminous crops the evidence indicated that, in some cases the whole of the nitrogen had been taken up as nitric acid, but that in others that source seemed to be inadequate.

It has been further shown that, under otherwise parallel conditions, there was very much more nitrogen as nitric acid in soils and subsoils, down to a depth of 108 inches, where leguminous than where gramineous crops had for some time been grown. The indication was, that nitrification had been more active under the influence of leguminous than of gramineous growth and crop residue.

At the same time, comparing the amounts of nitrogen as nitric acid in the soil where the shallow rooting *Trifolium repens* had previously been grown, with those where the deeper rooting *Vicia sativa* had yielded fair crops, it was found that, down to a depth of 108 inches, the *Vicia* soil contained much less nitric acid than the *Trifolium repens* soil; and it was concluded that most, if not the whole, of the nitrogen of the *Vicia* crops had been taken up as nitric acid.

New results of the same kind, which related to *Trifolium repens* as a shallow rooting and meagrely yielding plant, to *Melilotus leucantha* as a deeper rooting and freer growing one, and to *Medicago sativa* as a still deeper rooting and still freer

growing plant, very strikingly illustrated and confirmed the result of the exhaustion of the nitric acid of the subsoil by the strong, deep-rooting, and high nitrogen-yielding Leguminosæ. Still, the figures did not justify the conclusion that the whole of the large amount of nitrogen taken up by the *Medicago* crops could have had its source in nitric acid. It was obvious that much nitrification takes place near the surface; but as the surface soil became even somewhat richer in nitrogen, it was clear that it had not been the primary source of the whole of the nitrogen taken up by the plants. The source of much of it must have been either the atmosphere, or the subsoil; and if the subsoil, and yet not wholly as nitric acid, the question arises, in what other form of combination?

In another experiment, where one leguminous crop—beans—had been sown for many years in succession, but had frequently yielded very small crops, and sometimes failed, and over the whole period had given an average of little more than 30 lbs. of nitrogen per acre per annum, the land was then left fallow for several years, after which, in 1883, barley and clover were sown. In that year, in 1884, and in 1885, about 300 lbs. of nitrogen were removed per acre, chiefly in the clover crops. This result was obtained—where another leguminous crop had to a great extent failed, where the surface soil had become very poor in total nitrogen, where there existed a very small amount of ready formed nitric acid to a considerable depth, and where the surface was unusually poor in nitrogenous crop residue for nitrification.

Further, not only had this large amount of nitrogen been removed in the clover crops, but the surface soil became determinably richer in nitrogen. Here again, then, the primary source of the nitrogen of the crop could not have been the surface soil itself. It must have been either the atmosphere, or the subsoil; and assuming it to be the subsoil, the question arises whether it was taken up as nitric acid, as ammonia, or as organic nitrogen?

The various results adduced could leave no doubt that nitric acid was an important source of the nitrogen of the Leguminosæ. Indeed, existing evidence relating to nitric acid carries us quantitatively further than any other line of explanation. But it is admittedly inadequate to account for the amounts of nitrogen taken up, either by the *Medicago sativa* on the clover-exhausted land, or by the clover on the bean-exhausted land.

Direct experiments were made to determine whether the nitrogen of the Rothamsted raw clay subsoils, from which it was assumed much nitrogen had been derived in some way, was susceptible of nitrification, provided the nitrifying organisms, and other necessary conditions, were present. It was found that the nitrogen of such subsoils, containing only between 0·04 and 0·05 per cent. of nitrogen, and not more than six or eight parts of carbon to one of nitrogen, was susceptible of nitrification. It was also found that nitrification was more active in leguminous, than in gramineous crop subsoils.

Although it was clear that the nitrogen of raw clay subsoils, which constitutes

an enormous store of already combined nitrogen, was susceptible of nitrification, provided the organisms are present, and the supply of oxygen is sufficient, the results did not indicate that these conditions would be adequately available in such cases as those of the very large accumulations of nitrogen by the *Medicago sativa* for a number of years in succession on the clover-exhausted land, or by the red clover on the bean-exhausted land.

The question arose—whether roots, by virtue of their acid sap, might not, either directly take up, or at any rate attack and liberate for further change, the otherwise insoluble organic nitrogen of the subsoil? Accordingly, specimens of the deep, strong, fleshy root, of the *Medicago sativa* were collected and examined, when it was found that the sap was very strongly acid. The degree of acidity was determined, and attempts were made so to free the extract from nitrogen so as to render it available for determining whether or not it would attack and take up the nitrogen of the raw clay subsoil. Hitherto, however, these attempts have been unsuccessful.

When this difficulty arose, it was decided in the meantime to examine the action on soils and subsoils, of various organic acids, in solutions of a degree of acidity either approximately the same as that of the lucerne root-juice, or having a known relation to it.

It was found that the weak organic acid solutions did take up some nitrogen from the raw clay subsoil, and more from the poor lucerne surface soil. But when solutions of only approximately the acidity of the root-sap were agitated with an amount of soil which it was thought would be sufficient to yield so much nitrogen as to insure accurate determination, it was found that the acid frequently became neutralised by the bases of the soil, and that less nitrogen remained dissolved after a contact of 24 hours, or more, than after only 1 hour. The strength of the acid liquids was therefore increased, and the relation of soil to acid diminished. More nitrogen was then taken up, and more after the longer than after the shorter period of contact. Still, on adding fresh acid solution to the already once extracted soil, a limit to the amount of nitrogen rendered soluble was soon reached.

Here again, the conditions of experiment in the laboratory are not comparable with those of the action of living roots on the soil, and the results obtained did not justify any very definite conclusion as to whether the action of the roots on the soil, by virtue of their acid sap, is quantitatively an important source of the nitrogen of plants having an extended development of roots, of which the sap is strongly acid.

Dr. G. LOGES has published the results of experiments in which he acted upon soils by pretty strong hydrochloric acid, and determined the amount of nitrogen taken up. One of his soils contained, however, 0·804, and the other 0·367, per cent. of nitrogen; whilst the surface soil of the lucerne plot at Rothamsted contained only about 0·125, and the subsoil, which is assumed to have yielded large quantities of nitrogen to the crops, little more than 0·04 per cent. Again, in the one case LOGES found 40 per cent., and in the other 22·6 per cent., of the total nitrogen taken up.

It is obvious, therefore, that such an action is not directly comparable with that of root-sap on a poor subsoil. LOGES concluded however that the substance taken up is an amide or peptone body.

MM. BERTHELOT and ANDRÉ have also published the results of experiments to determine the character of the insoluble nitrogenous compounds in soils, and of the changes they undergo when acted upon by hydrochloric acid. They found the nitrogen in the extract existed partly as ammonia, but in much larger proportion as soluble amides, and that the amounts obtained of both, increased with the strength of the acid, the time of contact, and the temperature. They also found that when the clear filtered acid extract is exactly neutralised by potash, one portion of the amide still remains soluble, whilst another is precipitated, showing that the amides rendered soluble constitute two groups. Such re-precipitation is quite in accordance with the results obtained in our own experiments, in which less nitrogen remained dissolved after 24 hours' than after only 1 hour's contact, when, with the longer period, the acidity of the extract became neutralised.

In the experiments of BERTHELOT and ANDRÉ, as in those of LOGES, the strength of acid used was much greater than in the Rothamsted experiments, and very much greater than is likely to occur in any root-sap. Further, the soil they operated upon was about 4 times as rich in nitrogen as the Rothamsted subsoils, and with the strongest acid, and a temperature of 100° C., nearly one-third of the total nitrogen of the soil was dissolved.

Still, the results of LOGES, and of BERTHELOT and ANDRÉ, are of much interest as confirming the supposition that the insoluble nitrogenous compounds in soils are, or yield, amide bodies, and as indicating the changes to which they are subject when acted upon by acids. Supposing, however, the acid root-sap so to act on the insoluble organic nitrogen of the soil, and especially of the subsoil, the question still remains, whether the amide rendered soluble is taken up as such, or undergoes further change before serving as food for the plant? It is seen that ammonia is an essential result of the reaction; and the further question arises, therefore, whether the liberated ammonia is taken up as such, or is first oxidated into nitric acid? Then, again, is the soluble amide subjected to further change, perhaps first yielding ammonia, and this again nitric acid? On this supposition we are again met with the difficulty as to the sufficient aëration of the subsoil.

Independently of much other evidence, our own direct experiments have shown it to be probable, if not certain, that fungi can utilise both the organic carbon and the organic nitrogen of the soil; though they seem to develop the more freely when the humic matters have not undergone the final stages of change by which the compound of so low a proportion of carbon to nitrogen as is found in raw subsoils, has been produced. As bearing on the question whether amides, rendered soluble within the soil, may be taken up as such by chlorophyllous plants the results of various experiments

of others, made to determine whether such plants can take up such bodies, and assimilate their nitrogen, have been considered.

Upon the whole it seems probable, that green-leaved plants can take up soluble complex nitrogenous organic bodies, when these are presented to them under such conditions as in water-culture experiments, and that they can transform them and appropriate their nitrogen. If this be the case, it would seem not improbable that they could take up directly, and utilise, amide bodies rendered soluble within the soil by the action of their acid root-sap.

In connection with the subject of the conditions under which the insoluble organic nitrogen of soils and subsoils may become available to chlorophyllous plants, some results of FRANK are referred to. He observed that the feeding roots of certain trees were covered with a fungus, the threads of which forced themselves between the epidermal cells into the root itself, which in such cases had no hairs, but similar bodies were found external to the fungus-mantle, which prolonged into threads among the particles of soil. FRANK concluded that the chlorophyllous tree acquires its soil nutriment through the agency of the fungus.

Such a mode of accumulation by some green-leaved plants, obviously allies them in this respect very closely to fungi themselves; indeed, it is by an action on the soil which characterises non-chlorophyllous plants, that the chlorophyllous plant acquires its soil-supplies of nutriment. But inasmuch as, in the cases observed, the action was most marked in the surface layers of soil rich in humus, and it is stated that the development has not been observed on the roots of any herbaceous plants, the facts so far recorded do not aid us in the explanation of the acquirement of nitrogen by deep and strong rooted Leguminosæ from raw clay subsoils. Still, in view of the office within the soil which is by some attributed to micro-organisms, and other low forms, the observations are not without interest.

It is admitted that existing evidence on the various points which have been referred to is insufficient to explain the source of the whole of the nitrogen of the Leguminosæ.

The question arises, therefore, whether the free nitrogen of the atmosphere is fixed, either by the plant, under the influence of electricity or otherwise, or within the soil, by the agency either of electricity or of micro-organisms? We believe that the results of BOUSSINGAULT, and those obtained in conjunction with the late Dr. PUGH at Rothamsted, are conclusive against the supposition of the fixation of free nitrogen by the higher plants, under conditions in which the possibility of electrical action, or of the influence of micro-organisms, is excluded. The following is a brief *résumé* of the more detailed account and discussion, given in Part II., of the recently published results and conclusions of others, from experiments for the most part made under such conditions as not to exclude the possibility of the influence of electricity or of micro-organisms.

2. *The Evidence relating to the Fixation of Free Nitrogen.*

In the experiments of M. BERTHELOT, in all of which the gains of nitrogen are comparatively small, they have in some cases been attributed to electrical action, and in others to the agency of micro-organisms within the soil.

M. BERTHELOT first showed that free nitrogen was fixed by various organic compounds under the influence of the silent electric discharge, at the ordinary temperature; and he suggested that such actions probably take place in the air during storms, and when the atmosphere is charged with electricity, organic matters absorbing nitrogen and oxygen. He also experimented with currents of much weaker tension, more comparable with those incessantly occurring in the air, and in all cases he found that nitrogen was fixed by the organic substance. The gains were in amount such as would explain the source of the nitrogen which be considers crops must derive from the atmosphere.

Subsequently, he found that free nitrogen was brought into combination by argillaceous soils, when exposed in their natural condition, but not when they were sterilised. He also found gain when the natural soils were enclosed. He considered the results showed that there was gain of nitrogen quite independently of any absorption of combined nitrogen; in fact that there was fixation of free nitrogen due to living organisms. He further considered that such gains, not only serve as compensation for exhaustion by cropping, &c., but explain how originally sterile argillaceous soils eventually become vegetable moulds.

He also made experiments on the fixation of free nitrogen by vegetable earth supporting vegetation; and he found that there was a gain about equally divided between the soil and the plant, the latter having taken it up from the soil, which he considers is the true source of gain.

The results obtained under the influence of the silent discharge in bringing free nitrogen into combination with certain vegetable principles, of course owed their special interest to the inference that thus free nitrogen might be brought into combination within the plant; but M. BERTHELOT now considers it doubtful whether the higher plants do bring free nitrogen into combination at all. Obviously, however, if there are organic compounds within the soil which have the power of bringing free nitrogen into combination under the influence of electricity, the soil may be the source, and yet the agent may be the feeble electric current. But, so far as it is assumed that free nitrogen is brought into combination in the atmosphere itself, the resulting compounds will be found in the air, and in the aqueous depositions from it; and the limit of the amount of combined nitrogen so available over a given area, in Europe at any rate, is pretty well known.

In conclusion, although it must be admitted that M. BERTHELOT carefully considered, and endeavoured to estimate, all other sources than free nitrogen, yet the conditions of risk and exposure to accidental sources of gain, in experiments in open

air, are such that results so obtained cannot of themselves be accepted without reservation. But the fact that he found distinct gains in experiments in closed vessels, and that he obtained negative results with sterilised soils, is certainly in favour of the conclusion at which he arrived.

M. JOULIE made numerous vegetation experiments in which the soils and the plants were, with certain precautions, exposed to the free air, and in which known amounts of combined nitrogen were supplied. He found very variable, but in some cases very large, gains of nitrogen. He considered that the variations of result were largely due to the varying conditions as to mineral-supply in the different experiments.

M. JOULIE concluded that microbes probably play an important part in the fixation of nitrogen. He did not think that his results were favourable to the supposition that the plants themselves effected the fixation. For the present he limits himself to the establishment of the great fact of the fixation of the free nitrogen of the atmosphere, leaving to the future the exact explanation.

It is to be observed that the large gains shown were chiefly with a polygonous plant, buckwheat, and not with plants of the leguminous family, which are reputed to be "nitrogen collectors."

To show the practical importance of the fixation of free nitrogen, M. JOULIE calculates what would be the gain per hectare according to some of his results. It may be confidently affirmed, however, that such gains as he so estimates, do not take place, either with or without vegetation, in ordinary soils, in ordinary practice.

Dr. B. E. DIETZELL made vegetation experiments, in which plants were watered with distilled water, the drainage was returned to the soils, and the pots and their contents were exposed to free air, but protected by a linen roof; a rich garden soil, containing 0·415 per cent. of nitrogen, was used, several different conditions as to manuring were adopted, and peas and clover were the subjects of experiment. Thus the plants were of the leguminous family; but notwithstanding this, there was, in no case, a gain of nitrogen. In one there was neither gain nor loss, and in all the others there was a loss, in some cases amounting to about 15 per cent. of the total nitrogen involved.

That there should be loss with a soil containing 0·415 per cent. of nitrogen, that is about three times as much as most ordinary arable soils, is not at all surprising; and it is seen that, neither from the combined nitrogen of the atmosphere, or that due to other accidental sources, nor from free nitrogen, either directly or indirectly, did these reputed "nitrogen-collectors" gain nitrogen to compensate the losses from the rich soil. Indeed, Dr. DIETZELL's results are quite accordant with well established facts.

Professor FRANK also made vegetation experiments in free air. His soil was a humus-sand, containing only 0·0957 per cent. nitrogen; distilled water was used for watering, and the vessels were deep and narrow cylinders, without any arrangement at the bottom for drainage, or for aëration.* In three experiments without a plant, in one with two lupins, and in one with one lupin and incarnate clover together,

* See foot-note at p. 61.

there was a loss of nitrogen; whilst in one with three lupins, and in one with one lupin there was a gain. FRANK considered it probable that where a loss was indicated with vegetation, there had nevertheless been a gain, but not enough to compensate the loss.

In another experiment, with a soil about 12 times as rich in nitrogen, and many times richer than ordinary arable soils, he found a loss, due mainly to evolution of free nitrogen; and referring to this result, he says that if such losses take place in ordinary agriculture there must be natural compensation.

In the experiments in the deep and narrow vessels, without drainage, and without plants to cause evaporation, movement, and aëration, loss by evolution of free nitrogen is only what would be expected. Such loss would also be expected in the two cases of loss with growth, in both of which there was admittedly decomposing organic matter. It was also to be expected in the very rich soil. But it is doubtful whether, in the two cases of gain with growth, and therefore movement within the soil, and aëration of it, there would be any loss. In none of the experiments with loss, however, were the conditions comparable with those of ordinary soils, under ordinary treatment, and the losses found cannot be taken as any indication of what takes place in ordinary practice. It is probable that in such practice the loss by evolution of free nitrogen is much less than is generally assumed in discussions of this subject. Doubtless there is, however, frequently considerable loss by the drainage of nitrates.

FRANK considers that, independently of direct evidence against the supposition that the gains were due to the absorption of combined nitrogen from the atmosphere, an objection to such a view is that it would not explain the circulation of nitrogen in nature; and his main conclusion is, that there are two actions going on within the soil, one liberating nitrogen, and the other bringing it into combination, the latter favoured by vegetation.

Upon the whole it would seem that the losses found by FRANK may be explained by the special conditions of the experiments themselves; whilst the gains, if not to be accounted for by sources of error incidental to experiments made in free air, can only be explained by fixation in some way.

The most remarkable of the results indicating the fixation of free nitrogen are those of Professor HELLRIEGEL and Dr. WILFARTH. HELLRIEGEL found that whilst plants of the gramineous, chenopodiaceous, polygonous, and cruciferous families required combined nitrogen to be supplied within the soil, papilionaceous plants did not depend on such soil-supplies.

Peas sometimes grew luxuriantly in washed sand with nutritive solutions free from nitrogen, but sometimes failed, root-nodules being developed coincidently with luxuriance, but not without it. But when to the non-nitrogenous sandy matrix a few c.c. of the watery extract of a rich soil were added, the luxuriance was always marked, as also was the development of the root-nodules. Lupins, however, failed when treated in the same way, but succeeded when seeded by a watery extract of a

sandy soil where lupins were growing well, and root-nodules were then abundantly produced.

The amounts of produce recorded seemed to leave no doubt that they contained much more nitrogen than was supplied in the seed; whilst the amount added in the soil-extract was quite immaterial. The negative result with Gramineæ, with peas under sterilised conditions, or in sand not seeded with rich soil-extract, and with lupins in sand not seeded, or seeded with the rich soil-extract, and, on the other hand, the positive result with peas in the seeded sand, and with lupins when the sand was seeded with an extract from a suitable soil, seemed to exclude the supposition of any other source of gain than the fixation of free nitrogen under the influence of micro-organisms; and at first HELLRIEGEL was disposed to connect the action with the root-nodules and their contents.

WILFARTH gave the results of a subsequent season's experiments, which fully confirmed those recorded by HELLRIEGEL, both as to the negative result with other plants, and to the positive result with Papilionaceæ. Peas grew luxuriantly when the nitrogen-free soil was seeded with the watery extract from any cultivated soil, but serradella and lupins only when seeded with an extract from soil where these plants were growing.

In four experiments with lupins nearly 50 times as much dry substance was produced, and nearly 100 times as much nitrogen was assimilated, with, as without, seeding with the soil-extract!

WILFARTH concluded that the Papilionaceæ can derive the whole of their nitrogen from the air, but that it is doubtful whether the root-nodules are connected with the fixation, though the results point to the agency of bacteria in some way.

In reference to these results, whilst it can hardly be said that there is any unsolved problem in regard to the source of the nitrogen of other than our leguminous crops, it must be admitted that in spite of all the investigations and discussions of the last 50 years, the source of the whole of the nitrogen of these crops has not been satisfactorily explained by results obtained on the lines of inquiry until recently adopted. Evidence obtained on new lines should therefore receive careful consideration; and there can be no doubt that in recent years cumulative evidence has been adduced indicating that certain chlorophyllous plants may avail themselves of nitrogen brought into combination under the influence of lower organisms; the development and action of which would seem in some cases to be a coincident of the growth of the higher plants to be benefited. But such a conclusion is of such fundamental importance that further confirmation must yet be demanded before it can be considered to be fully established.

So long ago as 1853, Professor EMIL VON WOLFF obtained 6 times as much dry produce of clover, grown in an ignited rich meadow soil, as in the same soil in its natural state. Thus, the increased growth, and the increased assimilation of nitrogen, took place in a soil not only nitrogen-free, but sterilised; so that, unless micro-

organisms were acquired during growth, the supposition of their influence in fixing free nitrogen would be excluded.

Much more recently WOLFF has made numerous experiments with oats, potatoes, and various Papilionaceæ, in river-sand; in some cases unwashed, and in some washed; in some without manure, in some with purely mineral manure, and in some with nitrate in addition. Accordantly with common experience, there was little increase in the oats or potatoes with mineral, but much with nitrogenous manure; and, on the other hand, with the Papilionaceæ there was very marked increase with the mineral manure, and but little more by adding nitrate. In the experiments with lupins, beans, and clover, in unwashed sand, the results indicated gain of nitrogen beyond that probably due to the nitrogenous impurity in the sand; but with sand-peas, grown in washed sand, which was assumed to be nitrogen-free, the gains from some external source were unmistakable.

As to the explanation, WOLFF does not suppose that free nitrogen is fixed by the plants themselves; nor does he favour the view that it was fixed by the agency of micro-organisms. The plants may take up combined nitrogen from the air by their leaves; but he thinks it more probable that combined nitrogen is absorbed from the air by the soil, and that free nitrogen is fixed within the soil under the influence of porous and alkaline bodies. He admits that it is not explained why cereals do not benefit by these actions as well as Papilionaceæ; and he suggests whether the greater evaporation from their leaves causes greater aëration of the soil.

Here, then, the gain of nitrogen by the Leguminosæ is explained in a very different manner from that assumed by other recent experimenters. It seems to us, however, that the undoubted fact that the Gramineæ, and other plants than the Papilionaceæ, do not benefit by the actions supposed, excludes the supposition that WOLFF's results with Papilionaceæ are to be so explained. It is true that neither in the growth of the clover in ignited soil, nor in that of the sand-peas in the washed sand, were the conditions such as would seem favourable for the presence, development, and agency of micro-organisms. But if, in the experiments in free air, there was no accidental source of combined nitrogen, it would seem that the influence of micro-organisms is at least as probable as that of the actions which WOLFF supposes.

Professor ATWATER made numerous experiments, both on the germination and on the growth of peas. In eleven out of thirteen experiments on germination more or less loss of nitrogen was observed. In all but one out of fifteen experiments on vegetation, there was a gain of nitrogen, which was very variable in amount, and sometimes very large. As a general conclusion, he states that in some of the experiments half or more of the total nitrogen of the plants was acquired from the air.

He considers that germination without loss of nitrogen is the normal process; that loss, whether during germination or growth, is due to decay, and therefore only accessory. Nevertheless, he goes into calculations of some of his own results, showing, by the side of the actual gains, the greater gains supposing there had been

a loss of 15 per cent. of nitrogen, and the still greater gains if there had been a loss of 45 per cent., as in an experiment by BOUSSINGAULT under special conditions. Further, he says that whilst actually observed gains are proof of the acquisition of nitrogen, the failure to show gain only proves non-fixation, if it be proved that there was no liberation. He suggests that the negative results obtained by BOUSSINGAULT and at Rothamsted may be accounted for by liberation ; though at the same time he recognises that the conditions of the experiments excluded the action of either electricity or microbes. We may remark that, in the experiments both of BOUSSINGAULT and at Rothamsted, any cases of decay were carefully observed, and the losses found explained accordingly ; and it may be confidently asserted that the conclusions drawn were not vitiated by any such loss. This specious objection, putting out of court all negative results, is, however, a very old one ; as also is the one resuscitated by ATWATER, that luxuriance must be forced to a certain degree to favour the fixation of free nitrogen. On this point we may state that the results obtained at Rothamsted were as distinctly negative when luxuriance was favoured by supplies of combined nitrogen as when it was not.

ATWATER concludes that his results do not settle whether the nitrogen gained was acquired as free or combined nitrogen, by the foliage, or by the soil. He considers, however, that, in his experiments, the conditions were not favourable for the action either of electricity or of micro-organisms ; and he favours the assumption that the plants themselves were the agents. Lastly, he considers the fact of the acquisition of free nitrogen in some way to be well established ; and that thus facts of vegetable production are explained, which otherwise remain unexplained. To this, and other points involved, we shall refer again in our concluding remarks.

Lastly, we have to summarise those of the results and conclusions of BOUSSINGAULT which bear upon the present aspect of the question of the sources of the nitrogen of vegetation. In his earlier experiments, as in those at Rothamsted, sterilised materials had been used as soils ; but in 1858 he commenced a series in which more or less of a rich garden soil was mixed with sand and quartz. In some cases the plants were grown in free air, and in others in closed vessels with confined air. In several cases there was more or less gain of nitrogen ; but the greatest gain was in an experiment with a lupin grown in a closed vessel. BOUSSINGAULT points out that it was the soil and not the plant that had fixed the nitrogen. The result was so marked that he repeated the experiment in 1859, when he obtained almost identically the same amount of gain as in 1858. He also put 120 grams of the rich soil into a shallow dish, moistened it with distilled water, and exposed it to the air as an experiment on fallow. The results showed a small gain of nitrogen.

BOUSSINGAULT further found that mycodermic vegetation went on in rich soil, and he considered the gains of organic nitrogen represented the remains of such vegetation ; whilst the fallow experiment indicated that the experimental plants had little to do with the action. His general conclusion was, that from the numerical results

it must be believed that the soil had fixed nitrogen; and he considered that, if there were not absolute proof, there was strong presumption, that the nitrogen of the air takes part in nitrification.

In the next year, 1860, he put into one large glass balloon a mixture of rich soil and sand, and into another a similar mixture with cellulose in addition; each was moistened with distilled water, and the vessels were then closed up for 11 years. During this period, without cellulose rather more, and with cellulose rather less, than one-third of the nitrogen of the soil was nitrified; but in neither case was there any gain of total combined nitrogen. There was, indeed, in both cases, a slight loss of nitrogen indicated. BOUSSINGAULT concluded that free nitrogen had not contributed to the formation of nitric acid.*

The later results of BOUSSINGAULT did not therefore confirm those he obtained in 1858 and 1859; and in answer to one of ourselves he wrote in 1876, that he was not aware of any irreproachable observation which established the reality of the fixation of free nitrogen by the soil. He further stated his belief that neither the higher plants, nor mycoderms, nor fungi (*champignons*), fix free nitrogen. He also maintained the same view in conversation in 1883.

BOUSSINGAULT'S very distinct final conclusion against the supposition of the fixation of free nitrogen within the soil, by the agency of the lower organisms, notwithstanding his own clear recognition in 1858 and 1859 of the possibility of such an action, points to the necessity for still further confirmation of the evidence of others on the point during the last few years; for it will be remembered that whatever other sources of error were possible, the experiments in question were made in closed vessels, and not in free air, with all the risks incident to experiments so conducted; and if there may have been error with such an experimenter, and under such conditions, caution should surely be exercised in accepting very important conclusions founded on results obtained for the most part under less favourable conditions.

3. *General Considerations and Conclusions.*

So much for the evidence of direct experiment as to whether the higher plants, or soils, by the agency either of micro-organisms or otherwise, fix the free nitrogen of the atmosphere. It is clear that since experimenting in free air instead of in closed vessels has become more general, there has been a great accumulation of evidence which is held to show the fixation of free nitrogen. But not only are the gains in

* Quite recently ('Compt. Rend.,' vol. 106, 1888, pp. 805 and 898) M. SCHLŒSING referring to these results says that for his part he was satisfied with this result of BOUSSINGAULT, and should not have entered upon new experiments, had not the question been recently taken up and answered in a contrary sense. He then gives the results of experiments in which he submitted various soils to the action of air in closed vessels, supplying oxygen as it was used up. The result was that the air of the vessels neither lost nor gained nitrogen. There was therefore no fixation.

some cases small, and in others very large, but the modes of explanation are so different, indeed so conflicting, that it seems essential to hold final judgment in abeyance for the present.

The various modes of explanation of the observed gains of nitrogen are:—that combined nitrogen has been absorbed from the air, either by the soil or by the plant; that there is fixation of free nitrogen within the soil by the agency of porous and alkaline bodies; that there is fixation by the plant itself; that there is fixation within the soil by the agency of electricity; and finally that there is fixation under the influence of micro-organisms within the soil. The balance of the evidence recorded, is undoubtedly in favour of the last-mentioned mode of explanation. Indeed, it seems to us that, if there be not experimental error, there is fixation within the soil, under the influence of micro-organisms, or other low forms.

Assuming that definite decision on the point must wait for further evidence and discussion, it will nevertheless be well, in the meantime, to consider the facts of agricultural production in their bearing on the question, with a view of forming a judgment as to how far the establishment of the reality of the fixation of free nitrogen, either by the plant or by the soil, is so essential for the solution of the problems which such production presents, as is by some supposed.

It has been seen that much of the investigation that has been undertaken in recent years, has been instigated by the assumption that there must exist natural compensation for the losses of combined nitrogen which the soil suffers by the removal of crops, and for the losses which result from the liberation of free nitrogen from its combinations under various circumstances. In some cases, however, the object seems to have been for the most part limited to an attempt to solve the admitted difficulty as to the explanation of the source of the whole of the nitrogen of the Leguminosæ.

As to the losses which the soil sustains by the removal of crops, BERTHELOT for example assumes that 50 to 60 kilog. of nitrogen will be annually removed from a hectare of meadow (= 45 to 54 lbs. per acre), and that as only 10 kilog., or less, of this will be restored as combined nitrogen in rain, &c., there will be an annual loss of from 40 to 50 kilog. per hectare (= 36 to 45 lbs. per acre); so that, if there were not compensation from the free nitrogen of the air, the soil would become gradually exhausted. Further, he considers that the fact of the fixation of free nitrogen, not only explains how fertility is maintained, but how argillaceous soils which are sterile when first brought into contact with the air, gradually yield better crops, and at length become vegetable moulds. FRANK again, assumes that the average loss of nitrogen by the removal of crops is 51 kilog. per hectare (= 45 lbs. per acre).

It is quite true, that a good hay crop may contain as much as 50 to 60 kilog. of nitrogen per hectare, but it may safely be affirmed that, in ordinary practice, even in the case of an unusually fertile meadow, such an amount is not annually removed for a number of years in succession, without the periodical return of manure supplying nitrogen; whilst, taking the average of soils, the annual yield will not reach the

amount supposed, even with the ordinary periodic return, and without such return gradual exhaustion would be very marked. Indeed, it is well known that there is no more exhausting practice than the annual removal of hay without return of manure; so that, in point of fact, restoration in anything like the degree supposed certainly does not take place. Next to the removal of hay, the consumption of grass for the production of milk is the most, but still very much less, nitrogen-exhausting; whilst if the grass be consumed by store or fattening animals, the loss is very much less still; indeed it is very small.

Obviously, however, it is more important to consider, what is the probable average loss of nitrogen over a given area by the removal of crops generally, and not by that of grass alone. Moreover, in making such an estimate it is not the total nitrogen of the crops that has to be reckoned; but, taking into account the return by manure, only the amount eventually lost to the soil. With the great variation according to circumstances, it is of course very difficult to estimate this at all accurately; but we may state that two independent modes of estimate lead to the conclusion that, for Great Britain for example, the average annual loss of nitrogen is more probably under than over 20 lbs. per acre (= 22·4 kilog. per hectare). In fact, the loss by cropping, under the usual conditions of more or less full periodical return by manure, is by no means so great as is generally assumed in discussions of this subject.

The loss of nitrates by drainage may, however, in some cases be considerable. There may also, under some circumstances, be loss from the soil by the evolution of free nitrogen. Such loss may take place in the manure heap, or in soils very heavily manured, as in market gardening, for example. But in ordinary agriculture such excessive manuring seldom takes place; and the soil is generally much poorer in nitrogen than in the cases of the experiments which have been quoted as showing great loss from rich soils. Loss may also take place when the soil is deficiently aërated; but here again the conditions of the experiments cited, in which considerable loss by evolution of free nitrogen was observed, are not the usual conditions of soils in actual practice. Indeed, the balance of evidence is against the supposition that there is a constant and considerable loss by the evolution of free nitrogen from arable soils which are only moderately rich in organic nitrogen, and which are fairly drained, either naturally or artificially. Some illustrations bearing upon this point will be found at pages 62-3.

Again, M. BERTHELOT thinks it probable, though not absolutely established, that there is loss of nitrogen from the plant itself during growth. Long ago, we ourselves supposed that there was such loss; but careful consideration of the evidence relating to the subject has led us to conclude that it is not proved, and to believe that it probably does not take place. It may be observed that when in his vegetation experiments M. BOUSSINGAULT found a loss of nitrogen, there was coincidently some decaying vegetable matter, such as fallen leaves; and in somewhat parallel experiments at Rothamsted, no loss of nitrogen was found as a coincident of growth, and in the

absence of dead vegetable matter. Indeed, if there were such loss during growth when there was no decay, either in M. BOUSSINGAULT'S experiments or in our own, it must have been almost exactly balanced by corresponding gain; an assumption which is without any proof, but which has nevertheless had its advocates.

In fact we conclude, that under the existing conditions of practical agriculture in temperate climates, the annual loss of combined nitrogen over a given area, by cropping and otherwise, is by no means so great as has been assumed; that the restoration required to compensate the loss is therefore correspondingly less; and further, that the known facts relating to the maintenance or the reduction of the fertility of soils, do not point to the conclusion that such loss as actually does take place, is compensated by such restoration.

The well-known accumulation of nitrogen which takes place in the surface soil within a few years, when arable land is laid down to grass, is, it may be admitted, not conclusively explained. At the same time, there is, to say the least, quite as much evidence in favour of the assumption of a subsoil, as of an atmospheric, source. At Rothamsted, for example, there is, in soil and subsoil, to the depth at which the action of some deep-rooted and large nitrogen-accumulating plants has been proved, a store of about 20,000 lbs. of already combined nitrogen per acre. It is true that whilst many other soils and subsoils will contain as much, or more, many will contain much less. Still, if further investigation should confirm the indications given in this and former papers, that in the case of the deep and strong rooting, and high nitrogen-yielding, Leguminosæ, much at any rate of their nitrogen probably has its source in the combined nitrogen of the subsoil, and that the accumulation in the surface soil is due to nitrogenous crop-residue, the nitrogen of which has come from the subsoil, it is obvious that a like explanation would be applicable to the accumulation which takes place when arable land is laid down to grass, including herbage of various root-ranges, and various habits of root-collection.

Then, again, as to the supposition that the gains of nitrogen in argillaceous matters of very low initial nitrogen contents, which gains are attributed to the fixation of free nitrogen, serve to explain the gradual formation of vegetable soils, there cannot be any doubt that, so far as nitrogen is concerned, the natural fertility of most soils is due to the accumulation of ages of natural vegetation with little or no removal of it, by animals or otherwise. If the amounts of nitrogen even now brought into combination over a given area under the influence of electricity in Equatorial regions, were not exceeded in the earlier periods of the history of our globe, that would be quite sufficient, with growth and with little or no removal, through the ages which modern science teaches us to reckon upon, for the ascertained accumulations in natural prairie, or forest lands; and it is these which have to a great extent furnished us with our meadows and pastures, and arable soils. Frequently the natural forests have been on the more elevated, or the more undulating lands, and the soils they have formed

are less rich than the prairie lands, for the most part found in the valleys or on the plains. Taking the vast areas of fertile natural prairie on the American continent, for example, sometimes of several feet in depth, it may be estimated that, in such cases, each foot of depth will contain from 6000 to 10,000 lbs., or even more, of combined nitrogen per acre (= 6720 to 11,200 kilog. per hectare); and the probable time of these accumulations entirely obviates the necessity of calling in the aid of the free nitrogen of the atmosphere, brought into combination either under the influence of the plants themselves or of micro-organisms within the soil.

Further, the history of agriculture so far as it is known, indicates that soils under cultivation without supplies by manure from external sources, do, as a matter of fact, gradually become less fertile. This, as a rule, will take place more rapidly in undulating or high forest lands, than in the natural grass or prairie lands of the plains.

Again, if we compare the amount of nitrogen in the surface soil of permanent grass land, with that of adjoining land of the same original character, but which has for some time been under arable culture, we find that the latter is much poorer in nitrogen. In illustration, it may be stated that whilst the surface soil of the grass land at Rothamsted contains from 0·25 to 0·30 per cent. of nitrogen, that of the corresponding arable land only contains from 0·1 to 0·15 per cent. The arable soil has, in fact, originally been covered with natural vegetation of some kind, with comparatively little removal, and consequent accumulation; whilst, under arable culture, much of the accumulated nitrogen has been used up, and the loss has not been compensated by free nitrogen brought into combination, under the influence either of electricity, or of organisms within the soil. Whether or not there is any restoration of the kind supposed, we believe that a consideration of the origin of soils generally, and of the history of agriculture in different countries, will lead to the conclusion that the losses of combined nitrogen by cropping, and in other ways, are not compensated by corresponding amounts of free nitrogen constantly brought into combination.

The Rothamsted field experiments have indeed now been continued long enough to afford some pertinent examples bearing upon this subject.

Thus, in the case of the fields under continuous wheat, continuous barley, alternate wheat and fallow, and continuous root-crops, the average annual yield of nitrogen in the crops with mineral, but without nitrogenous manure, has only been about or under 20 lbs. per acre (= 22·4 kilog. per hectare); the amount has declined to less than the average in the later years, and, coincidently with the continuous and diminishing growth, the percentage of nitrogen in the surface soil has been considerably reduced. The loss by the removal of even such small crops, together with that by drainage, has, therefore, as a matter of fact, not been compensated by free nitrogen brought into combination, either by the plants, or within the soil.

In a field where the leguminous crop, beans, had been grown 25 years out of 32, with mineral but without nitrogenous manure, and had yielded less than average agricultural crops, the percentage of nitrogen in the surface soil was also greatly reduced.

In another field, where the leguminous crop, red-clover, had been sown 12 times in 30 years, the clover failed many times, the yield of nitrogen in the crops very greatly diminished, and the percentage of nitrogen in the surface soil was greatly reduced.

Again, in a rich garden soil, where red clover has been grown for more than thirty consecutive years, and has yielded throughout good, but gradually much diminishing crops, it was found, after the first 22 years, that the nitrogen in the surface soil had been reduced from 0·5095 to 0·3634 per cent., calculated on the soil dried at 100° C.

Even in an actual course of rotation, of turnips, barley, clover or beans, and wheat, with mineral, but without nitrogenous manure, the percentage of nitrogen in the surface soil has been much reduced; whilst in a parallel rotation in which fallow takes the place of the clover or beans, the reduction is still greater.

Thus, in all the cases cited, including gramineous, cruciferous, chenopodiaceous, and even leguminous crops, and a rotation of crops, when each has been grown for many years in succession without nitrogenous manure, and has yielded comparatively small and declining amounts of nitrogen in the crops, there has, coincidently, been a considerable reduction in the amount of nitrogen in the surface soil. There has, in fact, not been compensation from the free nitrogen of the air, or at any rate not at all in amount corresponding to the annual losses.

Lastly, grass land which, under the influence of a full mineral manure, including potash, but without any supply of nitrogen for more than thirty years, has grown crops containing large amounts of comparatively superficially rooting leguminous herbage, succeeded by increased amounts of gramineous herbage, has, under those conditions, yielded about the same amount of nitrogen per acre as M. BERTHELOT assumes to be the average produce of a meadow; but it has done so only with coincident great reduction in the nitrogen of the surface soil.

Whether, therefore, we consider the facts of agriculture generally, or confine attention to special cases under known experimental conditions, the evidence does not favour the supposition that a balance is maintained by the restoration of nitrogen from the large store of it existing in the free state in the atmosphere. Further, our original soil-supplies of nitrogen are, as a rule, due to the accumulations by natural vegetation, with little or no removal, over long periods of time; or, as in the case of many deep subsoils, the nitrogen is largely due to vegetable and animal remains, intermixed with the mineral deposits. The agricultural production of the present age is, in fact, so far as its nitrogen is concerned, mainly dependent on previous accumulations; and as in the case of the use of coal for fuel there is not coincident and corresponding restoration, so in that of the use or waste of the combined nitrogen of the

soil, there is not evidence of coincident and corresponding restoration of nitrogen from the free to the combined state.

In the case of agricultural production for sale, without restoration by manure from external sources, a very important condition of the maintenance of the amount of nitrogen in the surface soil, or of the diminished exhaustion of it, is the growth of plants of various range and character of roots, and especially of leguminous crops. Such plants, by their crop-residue, enrich the surface soil in nitrogen. It is, as a rule, those of the most powerful root-development that take up the most nitrogen from somewhere; and this fact points to a subsoil source. But independently of this, which obviously might be held to be only evidence of the necessity of obtaining water and mineral matters from below, in amount commensurate with the capability of acquiring nitrogen from the air, the experimental results recorded in this paper can leave little doubt that such plants obtain at any rate much of their nitrogen from the subsoil. The question remains—whether or not the whole of it is derived from the soil and subsoil? At present it is not proved that it is. It is equally not yet conclusively proved that it comes from the atmosphere. It may be safely affirmed that, in the case of our gramineous, our cruciferous, our chenopodiaceous, and our solaneous crops, atmospheric nitrogen is not the source. If, therefore, it should be proved to be the source in the case of the Leguminosæ, it may be that the development of the organisms capable of bringing free nitrogen into combination within the soil is favoured by leguminous growth and crop-residue, as there can be little doubt is the case with those which induce nitrification.

Bearing in mind, however, the very large store of already existing combined nitrogen, especially in subsoils, it is obviously important to consider, in what way, or in what degree, this store may contribute to chlorophyllous vegetation?

There is in the first place the question, whether the roots of some plants, and especially those of certain deep and powerfully rooting Leguminosæ, whose root-sap is strongly acid, may either directly take up organic nitrogen from the soil and subsoil, or may attack and liberate it for further change, the nitrogen so becoming more available.

Again, so far as is known, the Fungi generally, derive their nitrogen largely, if not exclusively, from organic nitrogen. In the case of those of fairy rings for example, there can be no doubt that they take up from the soil organic nitrogen which is not available to the meadow plants, and that, on their decay, their nitrogen becomes available to the associated herbage. In the case of the fungus-mantle observed by FRANK on the roots of certain trees, it is to be supposed that the fungus takes up organic nitrogen, and so becomes the medium of the supply of the soil-nitrogen to the tree. More pertinent still, is the action of the nitrifying organisms in rendering the organic nitrogen of the soil and subsoil available to the higher plants. It may well be supposed, therefore, that there may be other cases in which lower organisms bring the

organic nitrogen of the soil and subsoil into a more available condition; whilst it seems not improbable that the growth and crop-residue of certain plants favour the development and action of special organisms. In conclusion we would submit that, whether or not it may eventually be conclusively proved that lower organisms have the power of bringing free nitrogen into combination within the soil, it would at any rate be not inconsistent with well established facts, were it found that the lower serve the higher, chiefly, if not exclusively, by bringing into a condition available to them, the combined nitrogen already existing, but in a comparatively inert state, in our soils and subsoils.

Postscript.

(Added October, 1888.)

As it seemed to us that, of the various results which have been considered relating to the fixation of free nitrogen, those of Hellriegel and Wilfarth are the most definite and significant, we decided to institute experiments on somewhat similar lines. We hoped to commence them early in the summer, but were not able to do so until the beginning of August. Decisive results cannot, therefore, be expected this season, but the experience gained will be of value in subsequent experiments.

This preliminary series comprises experiments with peas, blue lupins, and yellow lupins. The peas are grown (1) in washed sand, with the necessary mineral nutriment added, but with no supply of combined nitrogen beyond a small determined amount in the washed sand, and that in the seed sown; (2) in similarly prepared sand, but seeded with the extract from a rich garden soil; (3) in the rich garden soil itself. Each description of lupin is also grown—(1) in sand prepared as for the peas; (2) in the same washed sand, seeded with the extract from a sandy soil where lupins had grown luxuriantly; (3) in the lupin sandy soil itself; (4) in rich garden soil. The pots are all arranged in a small greenhouse.

As the plants are still growing, no quantitative results are as yet available. It may be observed, however, that, so far as can be judged by the eye, there seems, in the case of the peas, to be somewhat more growth where the sand was seeded with the soil-extract, supposed to contain organisms, than where it was not; whilst in the cases of the lupins there is apparently even somewhat less growth with than without the sandy soil-extract. Both with the peas and the lupins, the growth is very much more luxuriant in the garden soil; and in the case of the yellow lupins it is almost as luxuriant in the sandy soil in which lupins had grown, as in the garden soil. These first experiments can obviously be only considered as initiative; but it is intended to analyse the products in due course, and to undertake a new series earlier in the season next year.